Results ERNDIM QC scheme for CDG screening 2012

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Please find below the results of the 2012 ERNDIM scheme for CDG screening. For the current scheme, 59 participants registered from many countries around the world. We have shipped 8 samples, divided over 2 reporting periods. We offered the opportunity to order 2 vials per sample, corresponding to 50 µl plasma, for HPLC or CE using laboratories. For CE, interpretable profiles were obtained for most samples, while for HPLC, the sample volume was relatively low. The amount as requested in routine HPLC analysis (up to 250 microliter) is impossible to obtain in sufficient quantities of patients with abnormal glycosylation. Still, we hope that this scheme is of added value to most laboratories.

To ensure future rounds of this CDG scheme, I would to ask you if you could provide patient material, preferably plasma/serum without additives. Please, send samples (~3.0 ml) to my address below, including information about age, sex, and a brief clinical description on first visit of the patient. In this way, we could again deliver 8 samples for 2013 and offer 50 µl samples for CE/HPLC laboratories.

In case of any questions, please do not hesitate to contact us.

With kind regards, also on behalf of Cas Weykamp,

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General comments

From 59 participating centres, we have received 49 report forms for Round 1 (83%) and 44 report forms for Round 2 (75%). No sample degradation was reported, but some centres report on possible interfering substances. We are aware of some EDTA in the control samples, which could interfere with CE analysis. For isofocusing, we are not aware that EDTA can interfere and lead to disturbed glycosylation profiles, as suggested by some labs for samples 017 and 023. Isofocusing was employed most often (31), followed by HPLC (8), CE (7), mass spectrometry (2) and western blot (2).

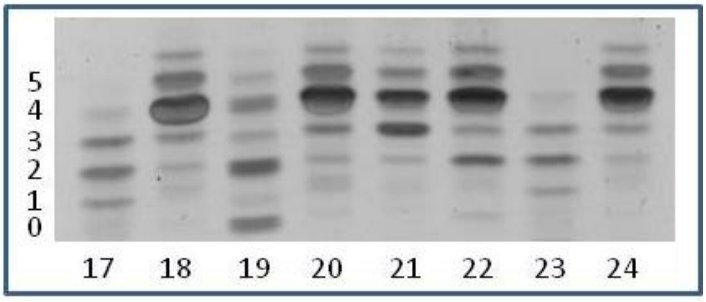
The shipped samples were from true CDG patients, from controls and of a blood sample containing neuraminidase (=sialidase) as secondary cause of abnormal transferrin glycosylation profiles (CDG 017 and CDG 023). In order to avoid further degradation of this sample during shipment and sample handling, leading to irreproducible results between laboratories, we inactivated the sialidase by short heating. The amount of transferrin in this sample was rather low, which caused problems for HPLC and CE analyses. For one of the control samples, we combined plasma of several individuals to have sufficient amounts for a duplo (samples CDG 018 and CDG 024).

Results

The final results of the eight samples with respect to CDG are summarized in the Table below.

Sample	**Clinical information (sex, age, phenotype)**	**Diagnosis**
CDG 017	M, 11 yrs, mental retardation, coagulopathy,	Presence of neuraminidase in the sample
CDG 018	M, 3 yrs, hepatomegaly	No CDG
CDG 019	F, 4 months, inverted nipples, hypotonia, cardiomyopathy, vomiting	PMM2-CDG
CDG 020	F, 5 yrs, strabismus, deafness, epilepsy	No CDG
CDG 021	M, 8 yrs, cutis laxa, mental retardation	Protein polymorphism
CDG 022	F, 9 yrs, mental retardation, ataxia, coagulopathy	PMM2-CDG
CDG 023	M, 8 yrs, frequent infections, liver fibrosis	Presence of neuraminidase in the sample
CDG 024	M, 2 months, thrombocytopenia	No CDG

Figure of transferrin isofocusing profiles:



In view of the problems for samples 017 and 023 as encountered by the CE/HPLC labs, we excluded these two samples for scoring for CE/HPLC labs. Since not all samples were reported, we expressed all scores as percentage of maximum possible score. The individual results of all centres are reported in the Table below. In agreement with ERNDIM rules, we applied a scoring system of 2+2:
Item C, technical aspects: 1 point for identification of abnormal profile and 1 point for identification of the profile as type I or II.
Item D, diagnostic suggestions: For normal profiles in general 2 points. For abnormal profiles, comments should be made on the possibility of secondary causes in view of the clinical indication. In addition, the right suggestions should be made for the next step in the diagnostic process that eventually will lead to the genetic defect. Scoring for this part is not so straightforward, but we tried to keep it as consistent as possible.

ERNDIM CDG017/CDG023

Item C. Isofocusing in almost all cases showed a clearly abnormal profile with increase of mono-, di- and trisialotransferrin and hardly any tetrasialotransferrin. This was correctly assigned as type II by most centres. The abundance of the disialo fraction was interpreted by some centres as suggestive for CDG type I. Although this can't be excluded fully on basis of an isofocusing profile, the presence of monosialo- and trisialo bands would favour an annotation of type II. EDTA was suggested to be present in the sample as disturbing factor for IEF. To our knowledge, EDTA does not lead to aberrant IEF profiles.
CE/HPLC results showed low signals in general. In such cases, it is indeed justified not to over interpret the data and ask for a repeat sample. In many cases, the profile was interpreted as abnormal. It was rather remarkable that many centres, especially using CE, did not correctly correlate the retention times with the individual sialotransferrin fractions. Although this could in part be related with the low signal intensity, the near absence of a tetrasialotransferrin peak could complicate this annotation. Since these type of samples occur in real practice, it is suggested to standardize the retention times in some way.

Item D. For these type of samples, suggesting the option of secondary causes is very important to prevent unnecessary diagnostic work. Especially for CDG023, where infections and liver disease are mentioned (both known causes of secondary type II abnormalities), this should be noticed. Some centres directly suggested MGAT2-CDG (CDG-IIa). Although there are similarities in profile type, it is important to follow the respective steps of excluding secondary causes and then additional diagnostics. For type II cases, it is justified to ask for a repeat sample, but in addition discuss the presence of secondary causes such as the presence of neuraminidase in the sample. As further diagnostics, ApoCIII could be mentioned to investigate the presence of an isolated N-glycosylation defect or a combined N- and O-glycosylation defect. In addition or alternatively, analysis of protein linked glycans could provide additional information. In only few cases (like for cutis laxa), the clinical information is sufficient to directly suggest a certain genetic defect (at this point in time).
Scoring item D (not for CE/HPLC labs); 1 point for suggesting secondary causes and 1 point for further diagnostics to identify the gene defect (at least 1 of the many options).

ERNDIM CDG018/CDG024

A normal profile was identified by almost all centres. Some centres suggested to repeat the analysis at later stage in view of the very young age of the patient. Indeed, it is known that CDG patients can present with normal transferrin glycosylation within the first 1-2 months of age. In view of the presentation of thrombocytopenia, some centres suggested to check for SLC35A1-CDG.

ERNDIMCDG019
A clear CDG type I profile was obtained for this patient by almost all centres. A transferrin polymorphism is not very likely and the clinical phenotype is not suggestive of either galactosemia or fructosemia as secondary causes of CDG type I. The clinical features are highly suggestive for PMM2-CDG (CDG-Ia).
Scoring item D: 1 point for discussing secondary causes, 1 point for further suggestions (at least PMM enzyme assay, additionally LLO analysis could be suggested). If a diagnosis of PMM2-CDG was suggested directly as most likely cause by referring to the clinical description, 2 points were scored.

ERNDIMCDG020
A normal profile was identified by almost all centres.

ERNDIMCDG021
This patient is not suffering from a confirmed CDG, but has a transferrin polymorphism. When using isoelectric focusing, an increase of trisialotransferrin was observed, more or less in equal quantities as tetrasialotransferrin. In most cases, this has been recognized as a possible polymorphism in the transferrin protein. Indeed, incubation of the sample with neuraminidase showed two bands, which is in agreement with a protein polymorphism. Alternative ways to exclude a polymorphism include analysis of parental samples. In CE analysis, an additional peak is seen at the position of disialotransferrin. This led to a suggestion of a type I profile in several cases, but in most cases the option of a polymorphism was not recognized. The same polymorphism did not reveal abnormal peaks in analyses by HPLC, western blot or mass spectrometry.
In view of the signs of cutis laxa, some centres suggested to perform ApoCIII. For ATP6V0A2-CDG patients (cutis laxa), transferrin glycosylation is normal within the first months of life (~6 months), while ApoCIII is already clearly abnormal. Although this is not known at older age, a very strong clinical suspicion justifies the extension of CDG screening by ApoCIII.
Scoring item D: suggestions in the direction of a polymorphism: 2 points. In addition, remarks could be made on further diagnostics if no polymorphism could be identified.

ERNDIMCDG022
A mild abnormality can be found in this sample with elevated disialotransferrin. Very few centres also report an increase of asialotransferrin. Secondary causes for a CDG-I profile should be discussed. The clinical phenotype could well fit with PMM2-CDG, but other subtypes are possible. Still, measurement of PMM activity seems the most logical next step.
Most centres identified a type I profile, although the diagnosis was missed by a few.
Scoring item D: 1 point for discussing secondary causes, 1 point for further suggestions (at least PMM enzyme assay, additionally LLO analysis could be suggested).

An overview of scores per individual sample and item and the overall scores are shown in the Table below.

	Technnical, Item C									Advice, Item D								
Sample ID	17	18	19	20	21	22	23	24	**% score of reported samples**	17	18	19	20	21	22	23	24	**% score of reported samples**
Averaged scores	1,8	2,0	1,9	1,9	2,0	1,8	1,8	2,0	96,2	1,2	2,0	1,6	1,8	1,8	1,3	1,2	1,9	80,7
1	1	2	1	2	1	0	1	0	50,0	0	0	0	0	0	0	0	0	0,0
2	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	2	1	2	87,5
3	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	1	1	2	81,3
4	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	1	1	2	81,3
5		2	2	2	2	2		2	100,0		2	2	2	2	2		2	100,0
6	0	2	2	2	2	2	0	2	75,0	0	2	2	2	2	2	0	2	75,0
7		2	2	2	2	2		2	100,0		2	2	2	2	1		2	91,7
8		2	2	2	2	2		2	100,0		2	2	2	2	1		2	91,7
9	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	1	1	2	87,5
10	1	2	2	2	2	2	2	2	93,8	1	2	2	2	2	2	1	2	87,5
11	2	2	2	2	2	0	2	2	87,5	2	2	1	2	2	0	2	2	81,3
12	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
13					2	2	2	2	100,0					2	2	2	2	100,0
14	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
15	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
16		2	2	2	2	2		2	100,0		2	2	2	0	1		2	75,0
17	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	2	1	2	87,5
18	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	1	2	2	93,8
19		2	2	2	2	2		2	100,0		2	1	2	2	1		2	83,3
20		2	2	2	2				100,0		2	2	2	2				66,7
21	2	2	2	0					75,0	0	2	0	0					25,0
22		2	1	2	2	2		2	91,7		2	2	2	0	2		2	83,3
23	2	2	2	2					100,0	1	2	2	2					87,5
24		2	2	2	2	2		2	100,0		2	1	2	2	1		2	83,3
25	2	2	2	2	2	2	2	2	100,0	1	2	1	2	2	1	1	2	75,0
26	2	2	2	2	2	2		2	100,0	1	2	1	2	2	0		2	71,4
27		2	2	2	2	2		2	100,0		2	1	2	2	1		2	83,3
28		2	2	2	2	2		2	100,0		2	1	2	2	2		2	91,7
29	2	2	2	2					100,0	1	2	2	2					87,5
30	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	1	1	2	81,3

31	2	2	1	2	2	2	2	2	93,8	0	2	0	2	2	0	0	2	50,0
32		2			2	2		2	100,0		2			2	1		2	58,3
33	2	2	2	2	2	0	2	2	87,5	0	2	1	2	2	0	0	2	56,3
34	2	2	2	2					100,0	0	2	2	2					75,0
35		2	2	0	2	2		2	83,3		2	1	0	2	1		2	66,7
36	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
37	2	2	2	2	2	2	1	2	93,8	2	2	2	2	2	2	0	2	87,5
38		2	2	2	2	2		2	100,0		2	1	2	2	1		2	83,3
39	2	2	2	2	2	2	2	2	100,0	2	2	2	1	2	1	2	2	87,5
40	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
41		2	2	2	2	2		2	100,0		2	2	2	1	2		2	91,7
42	1	2	2	2	2	1	1	2	81,3		2	1	2	0	0		2	58,3
43	2	2	2	2	2	2	2	2	100,0	1	2	1	2	2	0	1	2	68,8
44		2	2	2	2	2		2	100,0		2	2	2	2	2		2	100,0
45	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
46	2	2	2	2	2	2	2	2	100,0	1	2	2	2	2	1	1	2	81,3
47	2	2	2	2	2	2	2	2	100,0	2	2	2	2	2	2	2	2	100,0
48	2	2	2	2					100,0	1	2	2	2					87,5
49	2	2	2	2	2	2	2	2	100,0	1	2	1	1	2	1	1	1	62,5
50		2									2							