Summary

Congenital disorders of glycosylation (CDG) constitute a rapidly growing group of inherited diseases caused by defects in either synthesis (CDG type I, subtypes a-l), or processing (CDG type II, subtypes a-f) of the N-linked glycans of glycoproteins, resulting in proteins hypoglycosylation; the most common type is CDG Ia (>85 %). The clinical and biochemical picture of CDG is characterized by great variation in expression.

The diagnosis of CDG can be made by examination of N-glycans on any serum glycoprotein; most commonly used is transferrin (Tf). Carbohydrate deficient Tf (CDT) is relatively increased in CDG patients, and it thus serves as an indicator of this disease. Various methods are available for quantification of serum Tf isoforms. Decreased activity of appropriate enzymes in blood cells or tissues, topped by the identification of specific gene mutations, helps the CDG typing.

Aims of the study

The goals of this study were:

- Introduction of a screening method (IEF) for the diagnostics of CDG.
- Verification of the abnormal results by another method (e.g. HPLC).
- Introduction of an enzyme essay for phosphomannomutase (PMM), which is deficient in the most common type CDG Ia.
- Determination of the CDG frequency in our set of patients under clinical suspicion of a congenital metabolic defect.
- Presentation of an algorithm design of CDG screening.
- Presentation of my experience in the CDG screening of the paediatric population, suspected of having metabolic disease.

Materials and methods

A group of about 100 healthy individuals, and over 1100 patients, mostly with signs of a metabolic disease were examined. Beside these, several groups of patients with various chronic diseases have been screened out. Three CDG-positive sera that served as pathological reference samples obtained from other laboratories have also been checked out.

A common screening method based on IEF with direct immunofixation and Coomassie blue staining of serum Tf and α_1-antitrypsin (α_1-AT) has been selected. The procedure was accomplished by HPLC analysis (based on anion-exchange chromatographic separation of the individual Tf glycoforms on MonoQ/ResourceQ columns using gradient of Bis-Tris/NaCl buffers, and followed by photometric detection of the Fe-Tf complex at 460 nm), and by enzyme assay of PMM activity (followed spectrophotometrically, based on NADP(+) reduction to NADPH at 340 mm absorbency) in isolated leucocytes.

Results

I have introduced an IEF method of serum Tf. Apart from the Tf, also α_1-ATwas analysed, either separately or simultaneously on the same IEF gel. The method (originally intended for 52 samples) was adapted for lower series by use of smaller pieces of gel, thus allowing obtainment of results more quickly. Besides serum, also plasma, amniotic fluid, CSF, and serum/plasma/whole blood-dry spots have been checked out by IEF with good results. Distribution of isoforms of both glycoproteins in the controls and patient groups has been established.

I have introduced a HPLC procedure for verification of abnormal results obtained by IEF, and my experiences are described; in particular, HPLC system equipped with

detector of high sensitivity should be used, since not all detectors are suitable for this specific procedure.

I have established an enzyme assay in isolated leucocytes, and obtained the references values of PMM enzyme activity in controls and the family of our CDG patient.

Mild abnormalities of glycosylation (secondary, non CDG), detected in 7.2 % of our patients group have been associated with various, mostly pathological conditions, e.g. Hashimoto thyroiditis, systemic lupus erythematosus, epilepsy, hepatopathy, and cystic fibrosis.

Effect of long term treatment were found in two children treated by methotrexate or Phenaemaletten, and in an adult with combined therapy of carbamazepine, primidone and valproate; no effect of corticosteroids, antimalarics, or antibiotics, such as penicillin, or amoclen (trimethoprim) was noticed.

In this study, seven different phenotypes of Tf have been recognized. Only the variants C_1C_1 (in 86 %), and C_1C_2 (16 %) could be demonstrated among healthy subjects, while in a comparatively larger group of patients, apart from these two (in 78.7 % and 20 %, respectively), also the rare Tf C_2C_2 (0.6 %), and C_1C_3 (0.3 %), as well as heterozygous CB (0.2 % for Tf C_1B_{1-2}) and CD (0.1 % for both Tf C_1D_2 and Tf C_1D_{4-5}) phenotypes were found.

Apparently higher incidence of the Tf C_1C_2 subtype, noted in the group of patients suspected of an inherited metabolic disease (20 %; n=1100), and especially in two smaller groups of children with Crohn's disease (29.2 %; n=24) and cystic fibrosis (27.5 %; n=40), when compared to healthy controls (16 %; n=100), was not in fact, significant. No differences could be found in other subgroups of patients tested.

Since the Tf variants CB and Tf CD interfere with the usual IEF and also HPLC chromatography pattern, it is necessary to differentiate between the CDT and Tf variants by neuraminidase test, in addition to analysis of serum Tf in parents (carriers of the same genetic variant), which may help in suspicious cases.

I examined a 12-years patient showing a CDG-suspicious IEF pattern, who appeared to have a rare Tf C_1D_{4-5} protein variant, as proved by neuraminidase treatment, analysis of serum α_1-AT, and by investigation in the family of the affected adolescent boy.

α_1-AT was analysed in about 50 individuals. In addition to the most common phenotype MM, a variant MS has also been recognised in one patient.

I found out a 5.5-year old child that might correspond to CDG type IIx; he presented with dysmorphic features, mental retardation, partial agenesis of corpus callosum, and Ladd syndrome. Apart from Tf, also α_1-AT and TBG analysis revealed abnormal IEF-profile. The PMM activity in leucocytes showed normal results. The finding of an abnormal IEF pattern of apo C-III led to the suspicion of a combined N- and O-glycosylation defect. Further analyses of glycans for elucidation of the basic defect are pending.

Conclusion

CDG is a newly discovered metabolic disorder characterized by great diversity. So far, it is possible to distinguish 18 subtypes (some of them are treatable) with more than 600 patients described worldwide. Common CDG diagnostic methods are based on examination of N-glycans on any serum glycoprotein. Prenatal diagnosis is possible in all types of CDG for which the molecular defect is known.

This study describe 1) screening methods and algorithm of CDG diagnostic, 2) an overview of hypoglycosylation-findings in our set of investigated subjects, 3) a CDG patient with a rare combination of glycosylation defects, 4) distribution of Tf protein

variants, and their distribution in various diseases, 5) misguiding rare Tf variants detected, 6) observed association of some diseases / symptoms with increased CDT, 7) influence of some drugs by long-term treatment, 8) general pitfalls in CDG diagnostics, and 9) my practical experience having been acquired by 4years screening.