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The novel transferrin E592A variant impairs the diagnostics of congenital disorders of glycosylation

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ABSTRACT

Background: The analysis of serum transferrin either by high-performance liquid chromatography (HPLC) or isoelectric focusing (IEF) is the standard diagnostic procedure in patients with the suspicion of a congenital disorder of glycosylation (CDG). Carbohydrate-deficient transferrin (CDT) is also analysed in monitoring programmes in cases of alcohol abuse. We report a novel transferrin variant that impairs the analysis using conventional methods and propose alternative forms of analysis.
Methods: Transferrin samples were analysed using HPLC, immunoprecipitation followed by SDS-PAGE and IEF. Neuraminidase treatment followed by conventional IEF and electrospray ionization time of flight mass spectrometry (ESI-TOF MS) were applied before sequencing of the transferrin gene was performed.
Results: The novel transferrin variant E592A, found both in homozygous and heterozygous form, causes an altered charge of the transferrin molecule, which changes the results of IEF and HPLC and mimics an increase in trisialo-transferrin. The change in charge can be detected either by neuraminidase digestion followed by IEF or by ESI-TOF MS.
Conclusion: Conventional diagnostic methods for CDG are hindered by the novel transferrin E592A. Neuraminidase treatment followed by IEF and ESI-TOF MS can identify the mutation. The mutation appears to be functionally normal.

1. Introduction

Congenital disorders of glycosylation (CDG) are a group of inborn errors of metabolism which is divided into a vast variety of sub-entities with a broad spectrum of clinical manifestations. The most common variant by far is PMM2-CDG (formerly known as CDG-Ia) [1,2].

The standard diagnostic procedure in a case of suspected CDG is the analysis of serum transferrin (Tf) either by high-performance liquid chromatography (HPLC) or by isoelectric focusing (IEF). The transferrin molecule has two complex N-glycans attached to Asn432 and Asn630. The glycans are each terminated by two negatively charged molecules of sialic acid that allow discrimination using IEF/HPLC. In CDG affecting N-glycosylation, two typical patterns of cathodal shift in the IEF can be identified: pattern 1, which is typical for the subgroup CDG-I, is characterised by a decrease of tetrasialo-transferrin whereas di- and asialo-transferrin are typically elevated. Pattern 2, the typical pattern for CDG-II, shows an additional increase in the mono- and trisialo-transferrin bands [1] (Fig. 2).

Mutations in the transferrin gene are known to complicate the diagnostic process since they can alter the isoelectric point of transferrin which is detected using the aforementioned methods [3] (Fig. 3).

We present a novel mutation found in a consanguineous family which causes an altered IEF and HPLC pattern, thus leading to a seemingly increased trisialo-transferrin fraction mimicking CDG-II disease (Fig. 4).

The analysis of transferrin, in this context carbohydrate-deficient transferrin (CDT), also plays an important role in the monitoring of patients with chronic alcohol abuse. Mutations in the transferrin gene are known to impair the validity of these tests [4] (Fig. 5).

2. Materials and methods

The analysed samples were collected with informed consent from the probands/patients or their parents (Fig. 1).

Abbreviations: CDG, congenital disorders of glycosylation; Tf, transferrin; CDT, carbohydrate-deficient transferrin; HPLC, high-performance liquid chromatography; IEF, isoelectric focusing; ESI-TOF MS, electrospray ionization time of flight mass spectrometry; pK_a, acid dissociation constant.

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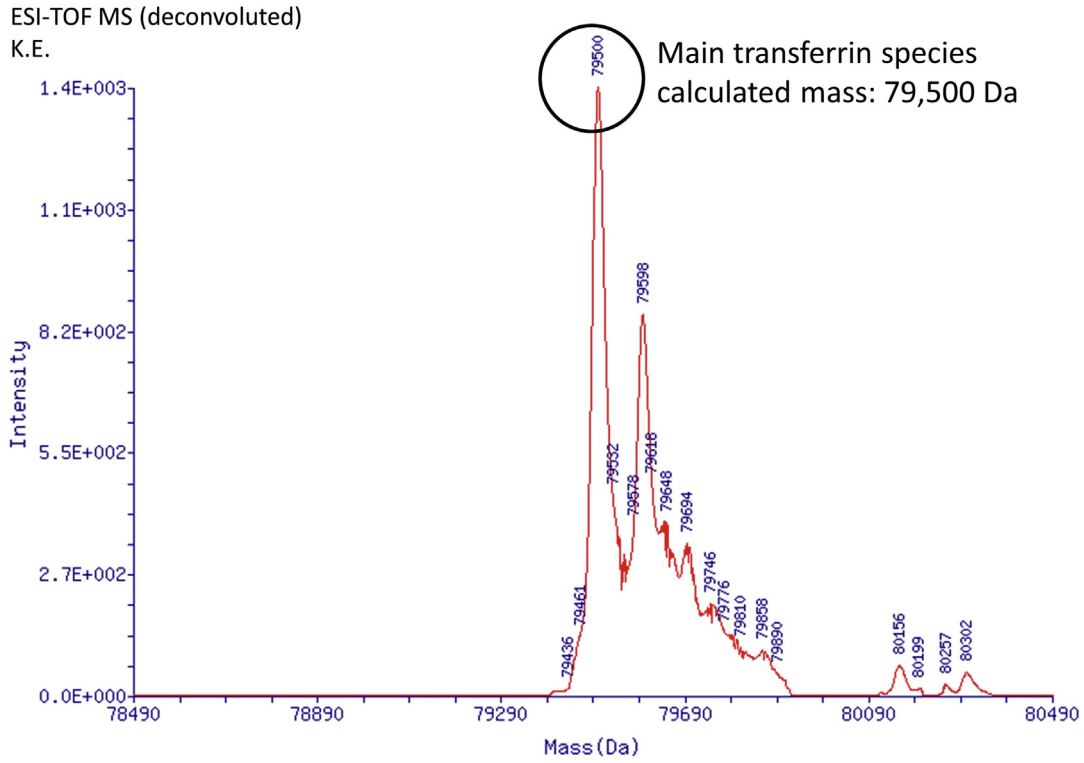

Fig 1. The pedigree of the affected family. M.E. and a dead sister both showed symptoms of an unidentified neurodegenerative disease. H.E., E.E., M.E. and C.E. are heterozygous for Tf E592A whereas K.E. is homozygous. Samples from the dead sister could not be obtained.

2.1. Isoelectric focusing and high-performance liquid chromatography

IEF and HPLC were performed according to the previously described methods [5,6].

2.2. Neuraminidase digestion followed by isoelectric focusing

In order to remove the sialic acids from the glycosylated transferrin molecule, 3 μl of serum were mixed with 11 μl of ultrapure water, 4 μl of 100 mM sodium acetate (pH 5.0) and 2 μl neuraminidase (Roche Diagnostics, Mannheim, Germany). The samples were incubated for 3.5 h at 37.0 °C. Afterwards, 1 μl Fe-III-citrate (0.1 M) was added. After 10 min of incubation at room temperature, 1 μl NaHCO (0.1 M) was added before IEF was performed according to the known protocol.

2.3. Immunoprecipitation and SDS-PAGE

The samples were prepared and SDS-PAGE was performed following the previously reported method [7].

2.4. Molecular mass measurement of transferrin (ESI-TOF MS)

Transferrin purified from patient K.E.'s serum using an immunoaffinity column coupled with anti-human transferrin IgG (Dako, Glostrup, Denmark) was injected into an Inertsil 300C4 short column

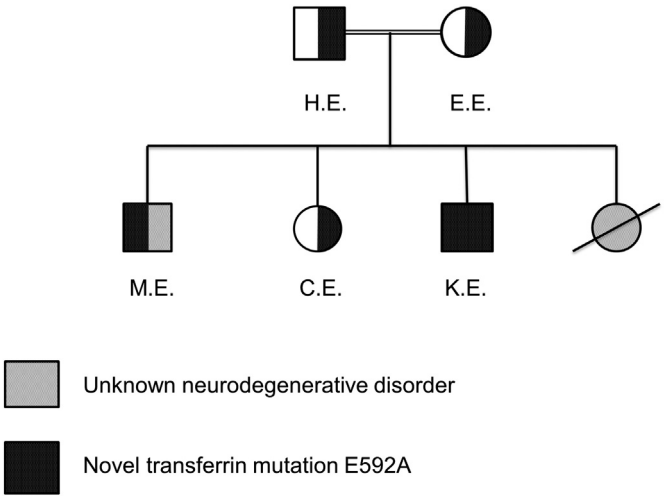

Fig 2. Isoelectric focusing of serum transferrin. Seemingly increased fractions of trisialo-Tf in all patients/probands. K.E. also shows signs of a reduced tetrasialo-Tf fraction.