Transferrin Allelic Variants May Cause False Positives in the Detection of Cerebrospinal Fluid Fistulae

Andrew J. Sloman and Robert H. Kelly

Variations in either the polypeptide sequence or the carbohydrate moieties of transferrin may result in altered electrophoretic mobility of this molecule. We report a case of an allelic (polypeptide) variant of transferrin with mobility similar to that of the $\beta_2$ (sialic acid-depleted) transferrin found in cerebrospinal fluid (CSF) and a few other body fluids. Allelic variation and other transferrin anomalies may be mistaken for the CSF isofom, resulting in false diagnoses of CSF fistulae.

Indexing Terms: genetic variants, electrophoresis, agarose gel

Allelic variants of transferrin may create separate transferrin bands in the $\beta$-globulin area on routine agarose gel electrophoresis (AGE) of serum, distinct from the typical transferrin band with $\beta_1$ mobility (1). In cerebrospinal fluid (CSF), an asialo version of transferrin produces a more slowly migrating band, i.e., $\beta_2$ mobility, which is not normally seen in serum. A CSF asialo isoform will exist for each allelic variant present in serum. Detection of such asialo isoforms in ear, nasal, and wound discharge is thus used to diagnose CSF or perilymphatic fistula (2-4). Here we report an uncommon genetic variant of transferrin initially discovered as four bands on AGE of CSF. Such a variant could be misinterpreted as a $\beta_2$ isoform when testing fluids for CSF leakage.

Case Report

A 45-year-old white woman underwent evaluation for multiple neurologic symptoms. This included serum and CSF protein electrophoresis as a screen for multiple sclerosis. Our protocol for subsequent analyses of these specimens was reviewed and approved by the Human Use Committee of our parent institution (University Health Center Hospitals of Pittsburgh).

Materials and Methods

Serum and CSF albumin, transferrin, and IgG concentrations were measured by rate nephelometry (Beckman Array; Beckman Instrument Co., Brea, CA) and the albumin ratio and IgG index were calculated (5). CSF was examined for oligoclonal immunoglobulins after 80-fold concentration by ultrafiltration (Minicon CS15; Amicon, Beverly, MA) and routine zone electrophoresis on agarose (6). Western blots were prepared with undiluted CSF and with serum diluted to contain an equivalent amount of transferrin. Proteins were again separated by AGE, then transferred to nitrocellulose by capillary blotting and probed with an anti-transferrin antisemur (Sigma, St. Louis, MO; cat. no. T6265) and a peroxidase-labeled anti-globulin (Boehringer-Mannheim, Indianapolis, IN; cat. no. 605-275). The chromagen was TMB/Blue (Transgenic Sciences, Worcester, MA).

Results

Serum AGE demonstrated an additional protein band migrating between transferrin ($\beta_1$) and complement C3 ($\beta_2$), whereas CSF AGE showed four bands with $\beta$ mobility (Figure 1). The albumin ratio (2.9) and IgG index (0.41) were both within the normal range for a 45-year-old individual (5). We performed immunofixation with anti-$\gamma$ chain, anti-$\alpha$ chain, anti-$\mu$ chain, and anti-light chain antisera to determine whether any of the $\beta$ bands could be identified as oligoclonal immunoglobulins. These studies were negative. Immunoblotting with anti-transferrin antisemur confirmed the presence of an allelic variant of transferrin, with four CSF bands representing the sialylated and asialo isoform of each allelic product (Figure 2).

Discussion

Transferrin is an iron-binding glycoprotein (79.5 kDa) with two N-linked carbohydrate side chains in the C-terminal domain. The glycan side chains exhibit microheterogeneity, although 80% of serum transferrin contains branched biantennary structures with four terminal N-acetyleneuraminic acid residues per molecule (7). The electrophoretic mobility of transferrin is influenced by its polypeptide sequence, carbohydrate side chain composition, and degree of iron saturation (4). Routine AGE does not resolve variations due to iron content, but genetic variants and molecules with altered composition of the glycan side chains do give rise to distinctive band patterns. The most common allelic variant (8, 9) of transferrin has been termed TFC (gene frequency 0.983) with several known subtypes (4). In general, faster-migrating variants (i.e., more anodic) have been given the designation TFB, and more slowly migrating variants (i.e., more cathodal) have been
abnormal

The AGE feature

Fig. 1. Agarose gel zone electropherograms of patient's serum (A) and CSF (B)

Arrow in A points to an abnormal serum band cathodic to $\beta_1$ transferrin. The CSF shows two abnormal bands: one (open arrow) has a mobility similar to the abnormal band in serum; the other (closed arrow) lies at the border between the $\beta$ and $\gamma$ regions.

Fig. 2. Anti-transferrin immunoblot of patient's CSF (A), control CSF (B), and patient's serum (C)

The patient's CSF shows four distinct transferrin bands, referred to as 1–4, proceeding from the anode to the cathode. Comparison with the control CSF immediately below allows the first band to be identified as the product of the most common transferrin allele, TIC, and the third band as its asialo isoform. Referring to the serum (C), the second band (open arrow) is shown to be a more slowly migrating allelic variant, TIF. The fourth band in the CSF (A, closed arrow) can now be identified as the asialo isoform of TIF. An atypical feature of the patient's CSF is the approximately equal concentration of the sialylated and asialo isoforms.

termed TFD. Individuals with a homozygous TfC genotype show a single transferrin band of $\beta_1$ mobility upon AGE of serum. Codominant expression of allelic variants in heterozygotes produces transferrin band doublets of equal concentration.

Alterations in the composition of the glycan portion of the molecule also affect electrophoretic mobility. Desialylation of the glycan side chains, for example, will retard electrophoretic mobility. Certain fluids such as CSF, aqueous humor, and perilymph normally contain an asialo isoform of transferrin with $\beta_2$ mobility. Examination of any of these fluids by AGE in an individual with a TfC genotype will reveal two transferrin bands, with $\beta_1$ and $\beta_2$ mobility, representing the sialylated and asialo isoforms, respectively. The latter may be present in smaller amounts than the fully sialylated molecule, giving rise to bands of different density. Corresponding analysis of these fluids in heterozygotes will reveal four bands, representing the sialylated and asialo forms of each allelic product.

Understanding the factors responsible for these unusual banding patterns is particularly important when fluids are examined for the presence of asialo-transferrin isoforms. The slowly migrating variant presented here has a mobility similar to that of asialo-TfC (i.e., $\beta_2$). Analysis of fluids from such a patient for asialo-transferrin by Western blotting could yield false-positive results. This can be avoided by simultaneous comparison of serum and CSF from the same individual. Analysis of sera from family members may also confirm a genetic variant (2).

Another potential pitfall is increased serum concentrations of partially desialylated transferrin isoforms, termed carbohydrate-deficient transferrin (10), which accompany chronic alcohol abuse. In such an instance, AGE of serum and CSF will reveal additional bands with intermediate electrophoretic mobility between $\beta_1$ and $\beta_2$, representing molecules with one or more sialic acid deletions.

Patients with cirrhosis, neuropsychiatric disease, and rectal cancer also reportedly have increased concentrations of asialo-transferrin, as do patients with a rare disorder of glycoprotein metabolism (10). Finally, normal aqueous humor contains asialo-transferrin (11), which may be important when examining fluids from head wounds.

In summary, variations of transferrin may cause difficulty in the interpretation of electrophoretic patterns of body fluids. This has been exemplified by an allelic variant that simulates the CSF isoform. Careful attention to clinical information and comparison of patterns in CSF, serum, and other fluids will allow the correct interpretation of unusual transferrin bands.

References