Urinary protein patterns in autism as revealed by high resolution two-dimensional electrophoresis

KATHERINE M. WILLIAMS and THOMAS MARSHALL
School of Pharmaceutical & Chemical Sciences, Sunderland Polytechnic, Sunderland SR2 7EE, U.K.

Autism is a pervasive neurodevelopmental disorder [1,2] now generally agreed to be biologically rather than psychologically caused [3]. Possible causes include genetic factors [4], early viral infection [5], slow viruses [6] and allergic responses [7]. Diagnosis is based on behaviour as there is no reliable biological marker. Previous studies have suggested that urinary protein profiles are characteristically modified in autism [8-10] and consequently we have compared urine from autistics and healthy controls using high resolution two-dimensional electrophoresis (2-DE) [11].

Early morning mid-stream urine was collected from autistics and age-matched controls and centrifuged (3,000 x g, 5 min) prior to Bradford protein assay [12,13] and 2-DE without dialysis or concentration [14,15]. Nine volumes of each urine was mixed with one volume of 0.625 M Tris HCl pH 6.8 containing 2% w/v sodium dodecyl sulphate (SDS) and two volumes of glycerol and heated at 95°C for 10 min. 2-mercaptoethanol was omitted from the sample preparation to retain the size distribution profile of most of the proteins. The samples were electrophoresed individually and also in pools (combining three individual autistic or control samples to give equivalent protein concentrations). 3 µg protein were analyzed by isoelectric focusing (IEF, first dimension) in 4% w/v polyacrylamide gel cylinders (65 x 3 mm) containing 9 M urea, 0.5% w/v Nonidet P-40 and 2% w/v Ampholine (pH range 2.5-4.0, 3.5-10.0 and 5.0-7.0, in a 2:6:3 v/v/v ratio) for 18 h at 300 V [14]. IEF was followed, without equilibration, by SDS-polyacrylamide gel electrophoresis (second dimension) on linear 4-25% w/v polyacrylamide gradient gels (75 x 75 x 3 mm) which were electrophoresed (75 mA/gel for 1.5 h) in precooled (4°C) 0.025 M Tris containing 0.2 M glycine and 0.1% w/v SDS [14]. Protein was detected by silver staining [15].

A representative 2-DE pattern of human urinary proteins is shown in Fig. 1. The chosen ampholyte mix gave optimal resolution of the predominant acidic components (pI 4.5-6.0). The individual urines displayed a wide variation in protein content (0.02 - 0.13 g/L) but the pooling of appropriate samples (as described above) overcome salt effects to give reproducible patterns which were more readily compared. Nevertheless, both the autistic and control groups showed wide variation in the intensity and distribution of individual protein spots. No consistent differences were detected between the two groups.

Previous studies have exploited gel filtration [8,9] and HPLC [10] to compare the urinary protein profiles of autistics and healthy controls. Whilst the findings have been contradictory a characteristic elevation of some urinary proteins in autism has been suggested. High resolution 2-DE [11] is currently the most powerful technique available for analysis of complex protein mixtures. The application of this technique to urinary protein analysis for comparison of autistics with healthy controls fails to detect significant differences between the two groups. This suggests that potential marker proteins, if they exist, must be of Mr < 10,000 which is below the working range (M, 10,000 - 1,000,000) of the standard 2-DE methodology.

Abbreviations: 2-DE, two-dimensional electrophoresis; SDS, sodium dodecyl sulphate; IEF, isoelectric focusing.