Visualization of patterned gene expression by MRI in the anterior zone of the cerebellum

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INTRODUCTION

The cortex of the cerebellum is histologically uniform region and its cytoarchitecture is similar through all lobules of the vermis and hemispheres. Underlying the cellular organization of the cerebellum, however, is a complex arrangement of parasagittal stripes and transverse zones that is revealed by using physiological mapping, anatomical tracing, mutational analysis, molecular markers, and cell degeneration [1, 2, and 3]. The primary organizer seems to be the Purkinje cell. The most extensively studied marker of cerebellar compartmentation is the antigen zebrin II (ZII), which is expressed strongly in subsets of Purkinje cells and forms a highly reproducible array of parasagittal stripes. Subsequent studies have shown that ZII to be an epitope on the respiratory isoenzyme aldolase C [1]. The functional significance of differential zebrin II/aldolase C expression is unknown, but it is clear that it reflects intrinsic differences between different Purkinje cell subsets rather than being a secondary response to patterns of usage. Cerebellar morphology is more complex in humans than in rodents and there is little information about intrinsic cerebellar patterning. In the present study, a specific focus has been placed on the anterior vermis of the human cerebellum by using anti-zebrin II immunohistochemistry because: 1- Significant abnormalities of the anterior vermis (lobules I-V) were observed among patients with chronic alcoholism. 2- In many species of mammals, the highly characteristic stripe topography of the anterior vermis is highly conserved, including in primates. Here, we compare ZII expression in the postmortem human anterior lobe vermis with MR data obtained from the same region. The results show an alternating pattern of high and low ZII expression, which has a clear correlation with the MR images.

METHODS

Adult wild type mice and homozygous BALB/c *npcnih* mice were obtained from Charles River Laboratories (St. Constant, PQ, Canada) and Jackson Laboratories (Bar Harbor, ME), respectively. Cerebellum samples for immunohistochemical study were obtained from cadavers of adult humans during the gross neuroanatomy laboratory courses, Faculty of Medicine, University of Calgary. Peroxidase immunohistochemistry was carried out on cerebellar sections and whole mounts as described previously [2]. MR image acquisition on fixed mice cerebella was performed on a 9.4-Tesla small animal MR scanner (Bruker BioSpin, Rheinstetten, Germany) using a home-built 2.5 cm loop gap resonator volume coil. Samples were immersed in *Fluorinert* to minimize background signals. T2w, T1w and proton-density MR images were obtained to assess their sensitivity to the parasagittal striping pattern. MR acquisition on a human cerebellum was performed at 3.0-Tesla (Signa; GE Healthacre, Waukesha, WI, USA) - with TR/TE=4090ms/109ms.

RESULTS

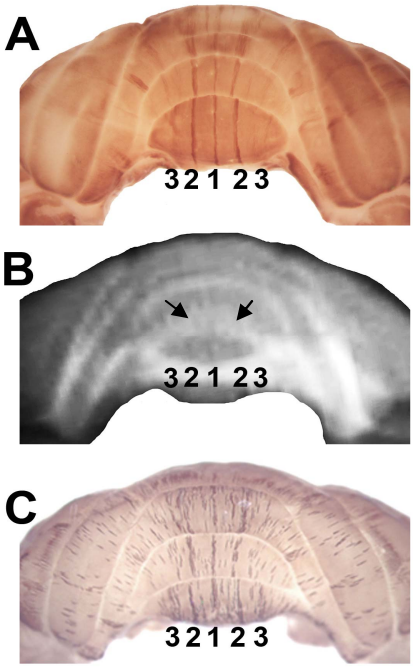
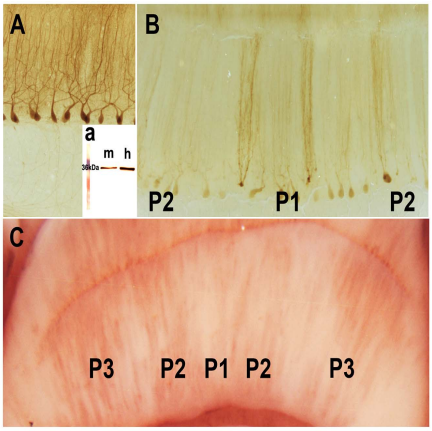


Fig 1: **A**) ZII immunohistochemistry of the adult mouse cerebellum shows parasagittal stripes in the AZ. **B**) MR image shows stripes in anterior zone of the adult mouse cerebellum fixed in 4% Paraformaldehyde. (T1w spin echo, TR/TE = 400ms/5ms, spatial resolution = 117 × 117 × 750 μm^3. **C**) CaBP (a pan-Purkinje cell antigen) immunostaining of the Niemann-Pick disease type C mutant mice (in which Purkinje cells die) also shows Purkinje cells degeneration is patterned stripes [3] that correspond to the ZII immunopositive stripes and the stripes on the MR images.

Fig 2: **A**) ZII is expressed strongly in Purkinje cells of the human cerebellum. **a**) Western blot of human (h) and mouse (m) cerebellar homogenates probed with anti-zebrin II. A single immunoreactive band is detected in both cases, apparent molecular weight 36 kDa. **B**) Subset of Purkinje cells in human cerebellum shows strongly expressed ZII and in other subset it is expressed weakly or nothing at all. **C**) Whole mount anti-zebrin II immunoperoxidase stained human cerebellum shows the array of stripes in the anterior zone vermis (for cerebellar stripe nomenclature, see [1]).



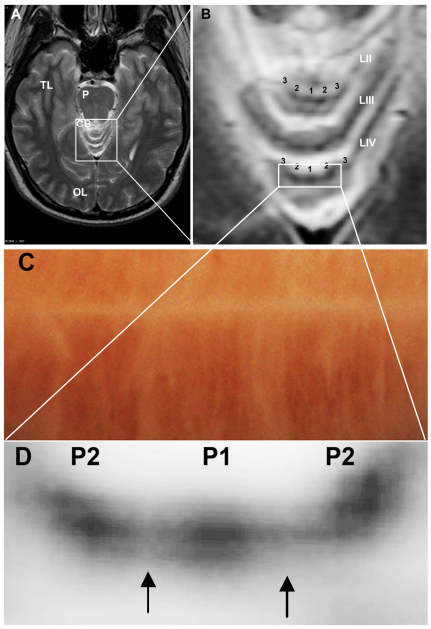


Fig 3: **A**) Axial T2-weighted image of the human brain (T2w, TR/TE=4090ms/109ms). **B**) Magnified view that reveals an alternating intensity pattern of stripes in the anterior zone of the cerebellum. The alternating MR signals intensity is probably due to the heavily (thick) and lightly (thin) myelination of axons in the white matter [see, 4, 5]. **C**) ZII immunohistochemistry, and **D**) highly magnified image from (A) showing the correlation of MR stripes to the ZII stripe.

DISCUSSION

This study demonstrates that the human cerebellum expresses the Purkinje cell antigen zebrin II. Two populations of Purkinje cells can be identified with high (P+) and low (P-) expression levels. The P+ and P- Purkinje cells are arranged in parasagittal stripes in the anterior zone. These stripes are not seen by using conventional histology. MR imaging of the anterior cerebellum reveals a similar stripe array to that seen with anti-zebrin II immunocytochemistry. Although the underlying cellular mechanism for the image contrast remains to be determined, there is a positive correlation between the molecular target and the MR images. This unique application of MR may be very useful for studying cerebellar abnormalities during development and the progression of cerebellum-related neurological diseases.

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