Supplementary Material

Methods

Mice and study approval

Gldc-deficient mice carried gene-trap alleles denoted Gldc\textsuperscript{GT1} (1) or Gldc\textsuperscript{GT2} (2) on a C57BL/6 background. Mthfr null mice were previously described (3). Mthfr/Gldc interaction studies were performed on a principally Gldc\textsuperscript{GT2} strain (but not fully isogenic) genetic background.

Mice were used for experimental matings from six weeks of age. Mice were maintained on a standard breeder diet (Teklad). Litters were generated by overnight matings and the day of finding a copulation plug was designated embryonic day 0.5 (E0.5). Animal studies were carried out under regulations of the Animals (Scientific Procedures) Act 1986 of the UK Government, and in accordance with the guidance issued by the Medical Research Council, UK in Responsibility in the Use of Animals for Medical Research (July 1993).

Histology

Fetuses were fixed overnight in Bouin’s solution (Sigma), dehydrated through an ethanol series, embedded in paraffin-wax, sectioned at 8 µm thickness and stained with haematoxylin and eosin.

Injection of the lateral ventricles

Pups at post-natal day 1 (P1; n = 16 pups from 4 litters) from 4 were chilled on ice and injected bilaterally with 2 µl of 0.4% trypan blue into each ventricles at a position 2/5 of the distance between the eye and Lambda as described (4). Pups were culled and the brain fixed in 4% paraformaldehyde and photographed prior to analysis of 1-2 mm tissue slices or 200 µm vibrotome sections.

Expression analysis

For mRNA and protein expression analysis, embryos were fixed in 10% formalin and dehydrated through an ethanol series prior to wax embedding and sectioning. In situ hybridisation was performed on sections using a digoxigenin-labelled anti-sense probe to Gldc (1). Immunostaining was performed using anti-Gldc (1:300; Atlas Antibodies, HPA002318) with anti-rabbit AlexaFluor secondary (Invitrogen Thermo Fisher, A11034) antibodies and DAPI staining of nuclei. Images were captured using Axiovision v4.8.2 (Zeiss) or µManager v1.4 (Open Imaging) software.
Statistical analysis was performed by Fisher-Exact test with p<0.05 considered significant, using Sigmastat (v3.5, Systat Software).

References


