

Supplemental figure 1.

(A, B) Genome comparison of the other human and mouse riboflavin transporter genes: *SLC52A2* (A) and *SLC52A1* (B). Note *SLC52A2* is another target gene of BVVL syndrome. (A) The surrounding genes on human *SLC52A2* are highly conserved on mouse chromosome 15. (B) The genes surrounding human *SLC52A1* are not overtly conserved on mouse chromosome 11. Red box on (B) is an enlarged view of the site corresponding to human *SLC52A1* on mouse chromosome 11. Two predicted genes *Gm12318* and *Gm12320* were found. (C) The gene tree of *SLC52A3* orthologues drawn by Treeview software was shown. (D) Conserved ratio of amino acid residues of human *SLC52A3* against vertebrates. Alignment search of human *SLC52a3* amino acid sequence was performed by NCBI (blastp), and ratio of conserved residues were calculated against 334 orthologues. Mutation points were shown as red bars. Predicted transmembrane regions were shown as blue bars. Between human riboflavin transporters and the orthologues, the surrounding sequences of each mutation point are highly conserved, suggesting these mutations would lead protein dysfunction or collapse of the 3D structures.

Supplemental figure 2

Identified *SLC52A3* mutations in BVVL patients and surrounding amino acid residues were aligned with main vertebrate orthologues genes and the other riboflavin transporters. Red-letter residues

mean identified mutations in BVVL patients. Green-letters mean assorted residues with human *SLC52A3*. Not only animal *SLC52A3* orthologues but also human *SLC52A1* and 2 conserved many mutation points, suggesting critical residues for correct folding and riboflavin transporting.

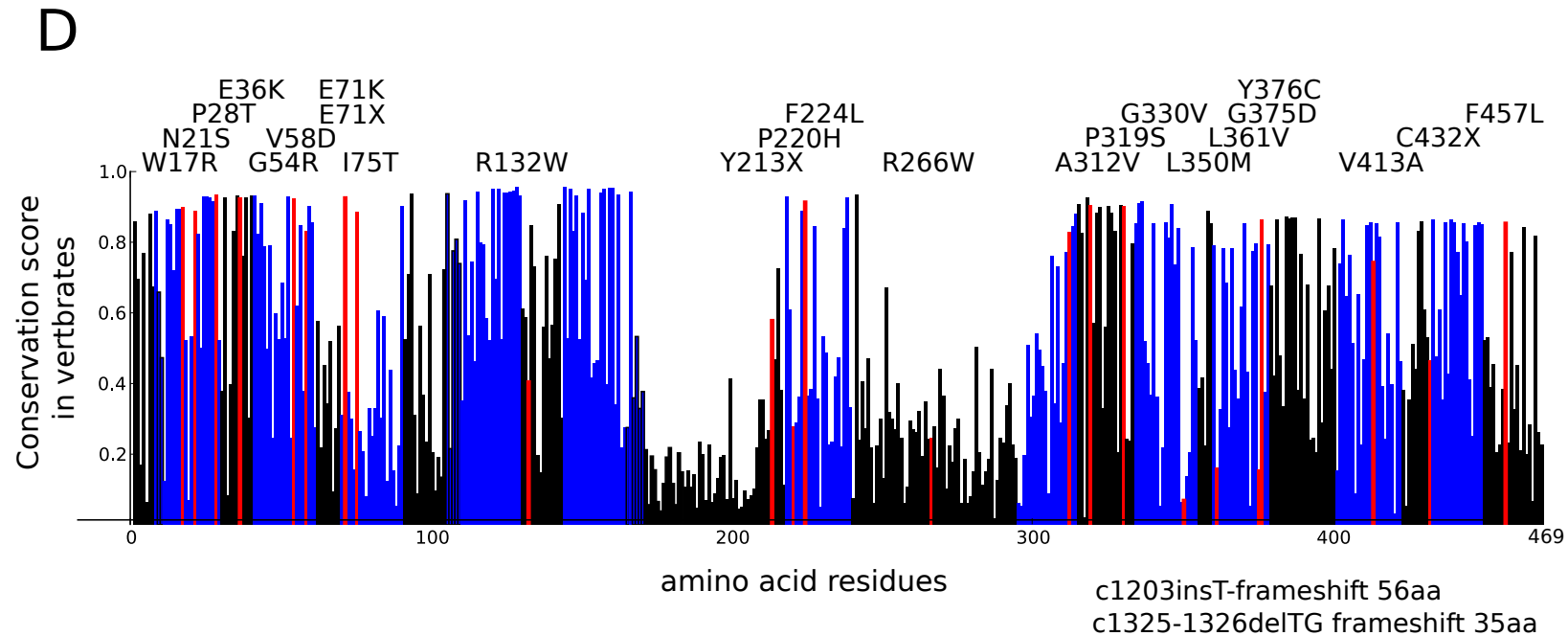
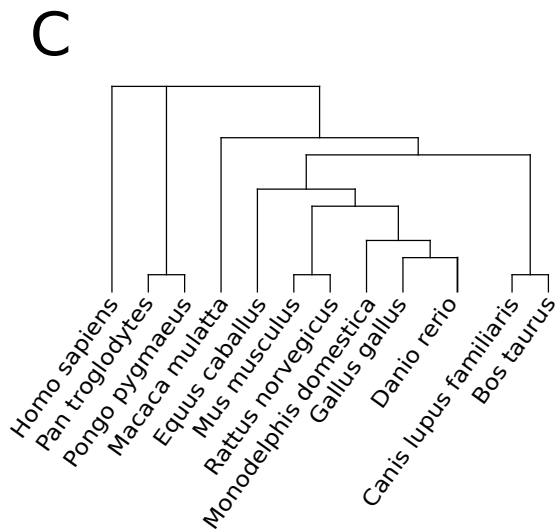
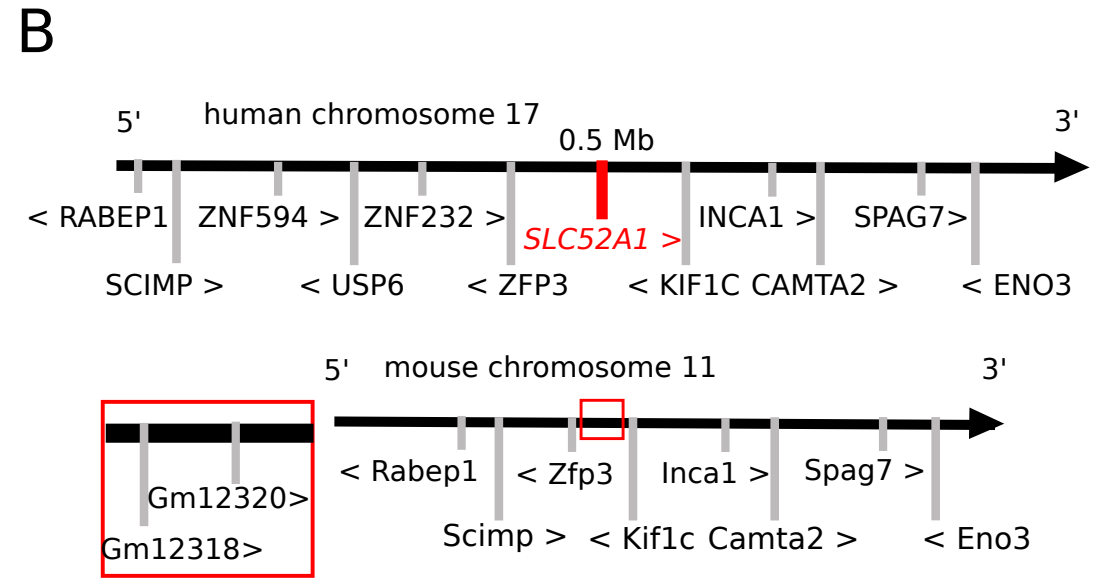
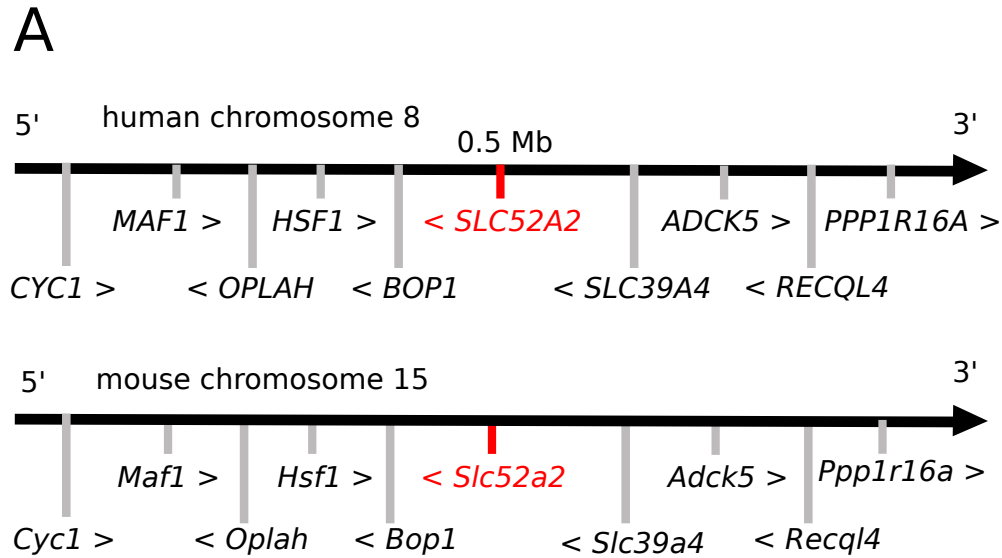
Supplemental figure 3

(A) Body weight gain in WT and heterozygous animals. Heterozygous mutant mice have no neural disordering phenotypes in 2years, and the growth rate is also normal comparing with WT. No homozygous mutants were born. (B) An example genotyping results. Samples were from E10.5 embryos by crossing *Slc52a3* +/- mice.

Supplemental figure 4

(A, B) RNA expression levels of putative human (A) and mouse (B) riboflavin transporters. . Note mouse *Slc52a3* expresses in both testis and placenta, though human *SLC52A3* is relegated in testis and *SLC52A1* in placenta. Riboflavin supplement in neural tissues is assumed with only *SLC52A2*.

Supplemental Figure 1



Supplemental Figure 2

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12                                     41
Homo sapiens SLC52A3 -FGMGSWVTINGLWVELPLLVMELPEGWYLP-
Pan troglodytes Slc52a3 -FGMGSWVTINGLWVELPLLVMELPEGWYLP-
Macaca mulatta Slc52a3 -FGMGSWVTINGLWVELPLLVMELPESWYLP-
Mus musculus Slc52a3 -FGMGSWVAINGLWVELPLLVTEELPEAWYLP-
Gallus gallus Slc52a3 -FGMGSWVAINGLWVELPLLVTVELPEQWDLP-
Danio rerio Slc52a3 -FGLGSWVSINGLWVELPLIVNVLPEGWDLP-
Homo sapiens SLC52A2 -FGMGSWAAVNGIWVELPVVVKDLPEGWSLP-
Homo sapiens SLC52A1 -FGMGSWAAVNGIWVELPVVVKDLPEGWSLP-

49                                     80
Homo sapiens SLC52A3 -QLANIGPLLVTLLHHRFRPSCLSEVPIIFTLLG-
Pan troglodytes Slc52a3 -QLANIGPLLVTLLHHRFRPSCLSEVPIIFTLLG-
Macaca mulatta Slc52a3 -QLANIGPLLVTLLHHRFRPSCLSEVPIIFTLLG-
Mus musculus Slc52a3 -QLANIGPLLVTLMHRFRPGCLSEVPVIFLILC-
Gallus gallus Slc52a3 -QMANVGPLFVTLMHRFWPGSLKEVVVIYVVS-
Danio rerio Slc52a3 -QFANLGPLLVTLAHKFCPGRLRENLAIYAVLS-
Homo sapiens SLC52A2 -ALGNLGLLVVTLWRRLAPGKDEQVPIRVVQV--
Homo sapiens SLC52A1 -ALGNLGLLVVTLWRQLAPGKGEQVPIQVVQVL-

127   137   208                                     229
Homo sapiens SLC52A3 -LPFMSRLPTYY- -SHLESRYLPAHFSPLVFFLLLLSI--
Pan troglodytes Slc52a3 -LPFMSRLPTYY- -SHLESRYLPAHFSPLVFFLLLLSV--
Macaca mulatta Slc52a3 -LPFMSRLPTYY- -SHLESRYLPAHFSPLVFFLLLLSV--
Mus musculus Slc52a3 -LPFMSQLPTYY- -WHQESRYLAPRFSPLLFFLLLLSF--
Gallus gallus Slc52a3 -LPFMMQLQAQY- -FRMETRYLPARFSTLIFFLLLMTA--
Danio rerio Slc52a3 -LPFMMQLPAKY-- -FIVETQYLPPNFSTEIFFSFLAV--
Homo sapiens SLC52A2 -LPFLSHLPPRF-- -DFLE------RFPASTFFWALTA--
Homo sapiens SLC52A1 -LPFLSHLPPPF-- -DFPE------RFPASTFFWALTA--

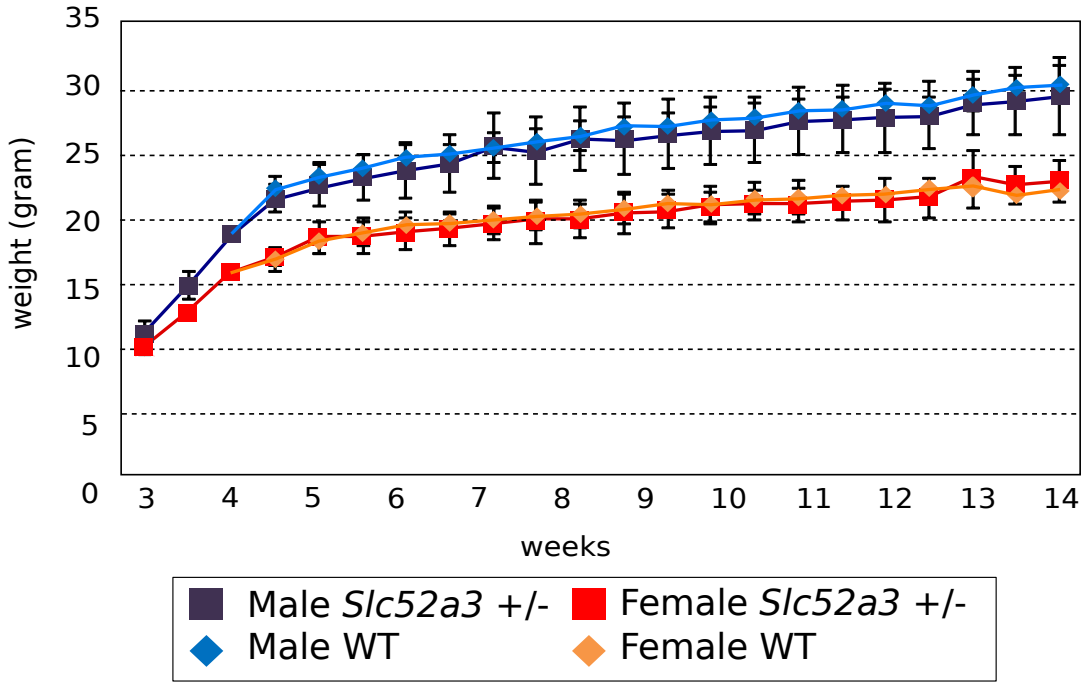
261   271   307                                     335
Homo sapiens SLC52A3 -TLHSIRLREEN- -VAFVNALTNGMLPSVQTYSCLSYGPVAYHL--
Pan troglodytes Slc52a3 -TLHSIRPREEN- -VAFVNALTNGVLPSVQTYSCLSYGPVAYHL--
Macaca mulatta Slc52a3 -TLHSIRPREEN- -VAFVNALTNGVLPSVQTYSCLSYGPVAYHL--
Mus musculus Slc52a3 -TLHSIRPRDTE- -VAFVNALTNGVLPSVQTYSCLPYGPVAYHL--
Gallus gallus Slc52a3 -ILNSFDQILED- -ITWVSSLTNGVLPSVQSYSCLPYGNTAYHL--
Danio rerio Slc52a3 -AVATVCRGLEG- -VLWVNSATNGLLPSVQTFSCMPYGNMAYHL--
Homo sapiens SLC52A2 -QVGAPGAEEEV- -LAATNALTNGVLPAVQSFSCLPYGRLAYHL--
Homo sapiens SLC52A1 -QLGSPGAEEEE- -MAFTSAVTNGVLPSVQSFSCLPYGRLAYHL--

345                                     381
Homo sapiens SLC52A3 -NPLASLVSMFLPNRSLLFLGVLSVLGTCFGGYNMAMA-
Pan troglodytes Slc52a3 -NPLASLVSMFLPNRSLLFLGVLSMLGTCFGGYNMAMA-
Macaca mulatta Slc52a3 -NPLASLLSMFLPNRSLLFLGVLSVLGTCFGGYNMAMA-
Mus musculus Slc52a3 -SPLACFLPIFLPNRSLLFLGVLTVLGTGFGAYNMAMA-
Gallus gallus Slc52a3 -NPLACIVAMVLPSRSLALLGTLSLAGTGFGAYNMAIA-
Danio rerio Slc52a3 -NPVACIIAMFFPKRSLVFLGILCLLGSTFFGGYNMAMA-
Homo sapiens SLC52A2 -NPLACFLAMGVLCRSLAGLGGLSLLGVFCGGYLMALA-
Homo sapiens SLC52A1 -NPLACFLAMGVLCRSLAGLVGLSLLGMLFGAYLMALA-

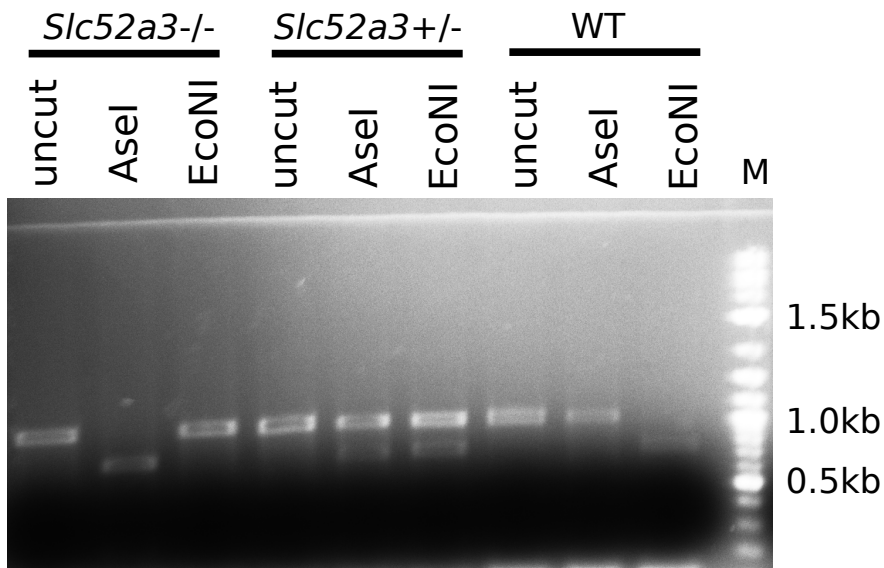
408   437   452   462
Homo sapiens SLC52A3 -GCLSYVKVMLGVVLRDLSRSALLWCGAAVQ- -NVLRLFSSADF--
Pan troglodytes Slc52a3 -GCLSYVKVMLGVILRDLSRSALLWCGAAVQ- -NVLRLFSSADF--
Macaca mulatta Slc52a3 -GCLSYVKVMLGVILRDLSRSALLWCGAAVQ- -NVLRLFSSADF--
Mus musculus Slc52a3 -ACLSYVKVMLGVILRDRSRSALLWCGAAVQ- -NVLKLFSSADY--
Gallus gallus Slc52a3 -----
Danio rerio Slc52a3 -GLLSYVKVMVGVILRDRSHSALVWCGAAVQ- -NVYHLFKSGDI--
Homo sapiens SLC52A2 -GVFSYVKVAASSLLHGGGRPALLAAGVAIQ- -SIYHVFHSRKD--
Homo sapiens SLC52A1 -CVFSYVKVAASSLLHGGGRPALLAAGVAIQ- -SIYHVFQSRKD--
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Supplemental Figure 3

A



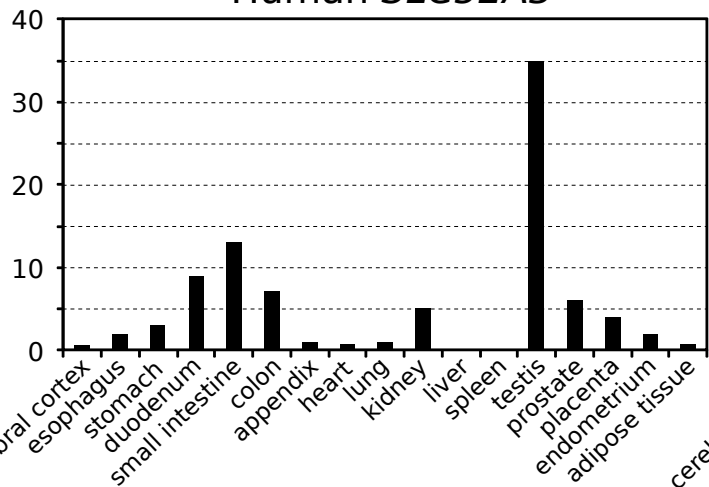
B



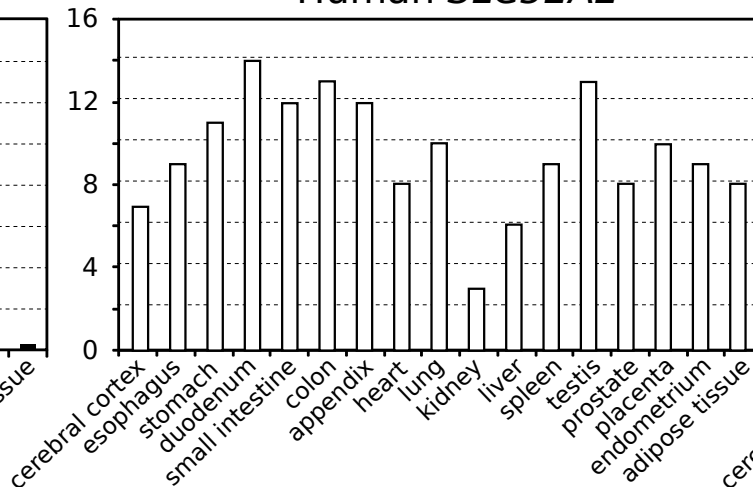
Supplemental Figure 4

A

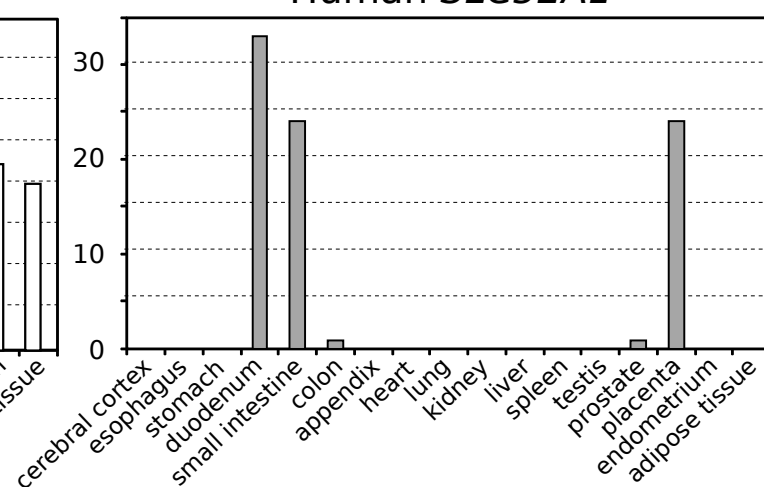
Human *SLC52A3*



Human *SLC52A2*

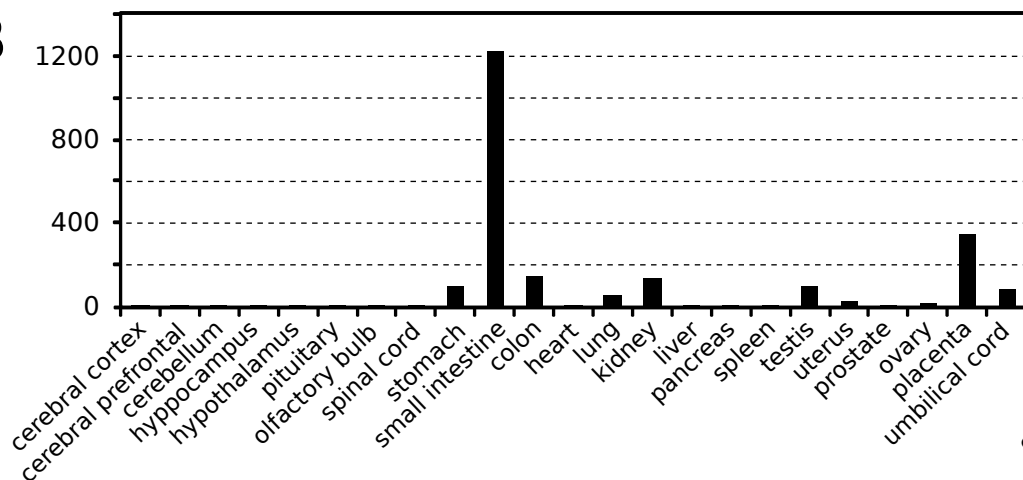


Human *SLC52A1*



B

Mouse *Slc52a3*



Mouse *Slc52a2*

