**Supplementary Information**

**Iron-related gene expression associated with magnetic susceptibility reductions in Gilles de la Tourette syndrome**

Ahmad Seif Kanaan1,2, Alfred Anwander1, Andreas Schäfer3, Riccardo Metere1, Torsten Schlumm1, Jamie Near4, Berkin Bilgic5, Jeremiah Scharf6, Kirsten Müller-Vahl2\* & Harald E. Möller1\*

1. *Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany*
2. *Department of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany*
3. *Siemens Healthcare GmbH, Diagnostic Imaging, Magnetic Resonance, Research & Development, Erlangen, Germany*
4. *Athinoula A. Martinos Center for Biomedical Imaging and Department of Radiology, Harvard Medical School, Boston, MA, USA*
5. *Douglas Mental Health University Institute and Department of Psychiatry, McGill University, Montreal, QC, Canada*
6. *Psychiatric and Neurodevelopmental Genetics Unit, Departments of Psychiatry and Neurology, Center for Human Genetics Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

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**Abbreviations & Symbols**

ADHD = attention deficit/hyperactivity disorder; AHBA = Allen Human Brain Atlas; ANTs = advanced normalization tools; BAI = Beck Anxiety Inventory; BDI-II = Beck Depression Inventory II; CAARS = Conners' Adult ADHD Rating Scale; CNR = contrast-to-noise ratio; CSF = cerebrospinal fluid; DN = dentate nucleus; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; EFC = entropy focus criterion; ESPIRiT = eigenvector-based self-consistent parallel imaging reconstruction (iterative); FBER = foreground-to-background ratio; FDR = false discovery rate; FLASH = fast low-angle shot; FSL = FMRIB software library; FWHM = full width at half maximum; GABA = γ-aminobutyric acid; GM = grey matter; GTS = Gilles de la Tourette syndrome; MADRS = Montgomery Asberg Depression Rating Scale; MNI = Montreal Neurological Institute; MP2AGE = magnetization-prepared 2 rapid gradient echo; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; OCB = obsessive-compulsive behavior; OCD = obsessive-compulsive disorder; OCI-R = Obsessive Compulsive Inventory—Revised; PC = principal component; PCA = PC analysis; PET = positron emission tomography; QI1 = quality index 1; QSM = quantitative susceptibility mapping; RN = red nucleus; ROI = region of interest; RVTRS = Rush Video-Based Tic Rating Scale; SHARP = sophisticated harmonic artifact reduction for phase; SN = substantia nigra; SNR = signal-to-noise ratio; STN = subthalamic nucleus; SVD = singular value decomposition; *T*1 = longitudinal relaxation time; TE = echo time; TKD = threshold k-space division; TR = repetition time; Y-BOCS = Yale-Brown Obsessive Compulsive Scale; YGTSS = Yale Global Tic Severity Scale; Δχ = relative magnetic susceptibility.

**1 Supplementary materials and methods**

**1.1 Clinical assessment**

All patients underwent a thorough clinical assessment battery (Gerasch *et al.*, 2016; Kanaan *et al.*, 2017) that included measurements of *(a)* tics using the Yale Global Tic Severity Scale (YGTSS) (Leckman *et al.*, 1989) and the modified Rush Video-Based Tic Rating Scale (RVTRS) (Goetz *et al.*, 1999); *(b)* obsessive compulsive behavior using the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Goodman *et al.*, 1989) and the Revised Obsessive Compulsive Inventory (OCI-R) (Foa *et al.*, 2002); *(c)* attention-deficit/hyperactivity symptoms using the DSM-IV symptom list for ADHD and the Conners' Adult ADHD Rating Scale (CAARS) (Conners *et al.*, 1999); *(d)* depression using the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) and the Beck Depression Inventory II (BDI-II) (Beck, 1961); and *(e)* anxiety using the Beck Anxiety Inventory (BAI) (Beck *et al.*, 1988).

**1.2 Measurement of serum ferritin levels**

A 10-ml blood sample was collected from the majority of the subjects (*N*=37) for the *in vitro* quantitative determination of serum ferritin as a representative measure of the body’s iron reserves. The sample was first centrifuged at 24,000 rpm for a period of 10 min to separate hematocrit from plasma, which was subsequently stored in 1000μl aliquots at −70 °C. Serum ferritin levels were quantified based on the electochemiluminescence immunoassay, in which a voltage applied to a sample containing tagged ferritin molecules induces chemiluminescent emissions that are measured by a photomultiplier (Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany).

**1.3 Masking of deep grey matter nuclei**

Masks of the striatum (caudate-putamen), globus pallidus and thalamus were obtained via the FSL-FIRST Bayesian model-based subcortical segmentation algorithm (18), which was applied on optimized hybrid-contrast MP2RAGE-QSM images (19, 20). Robust co-registration between skull-stripped MP2RAGE and FLASH data was achieved using rigid-body linear transformation of the *T*1-weighted data onto N4 bias-field-corrected FLASH magnitude data (http://github.com/stnava/ANTs). Given the difficulty of segmenting brainstem and cerebellar nuclei on *T*1-weighted data due to lack of contrast, in addition to the infeasibility of performing manual segmentation of multiple nuclei in many subjects, we utilized an atlas-based registration approach to achieve accurate delineations of brainstem/cerebellar nuclei. Specifically, the diffeomorphic greedy-SyN ANTs non-linear transformation model (http://github.com/stnava/ANTs) was used to compute a nonlinear transformation warp between MP2RAGE and MNI space, which was used to map each subject’s QSM data into standard space for subsequent calculation of a population-specific average image. The standardized QSM template exhibited high contrast in brainstem/cerebellar regions and was used to carefully delineate masks of the STN, SN, RN and DN (Fig. 1). All masks were delineated by the same operator and were subsequently warped back into native QSM space. The same atlas-based registration procedure was applied to obtain subject-specific masks of the lateral ventricles, which were used for referencing the QSM data to cerebrospinal fluid (CSF; median values). All masks were thresholded at 0.5 to ensure maximal inclusion of GM tissue while limiting partial-volume effects. Following visual inspection of all the masks for quality, median susceptibility values from all ROIs were computed for further analysis.

**1.4 Quality-control procedures**

To account for potential differences in the severity of motion-related image artifacts, we used a step-wise, multivariate outlier-detection approach implementing a robust Mahalanobis distance framework (15) to remove low-quality data based on *(a)* structural image quality indices calculated on the magnitude structural images and *(b)* susceptibility values extracted from subcortical nuclei. In general, Mahalanobis distance calculates how far each observation is to the center of a joint distribution, which can be thought of as the centroid in multivariate space. Robust distances are estimated from minimum covariance determinant estimators rather than the sample covariance. Data were regarded as outliers if the robust Mahalanobis distance was greater than the 97.5% quantile of the chi-square distribution (Supplementary Figure S1). In the first step, multivariate outliers were detected based on *(a)* the Shannon entropy focus criterion (EFC), which is an index for image ghosting and blurring (21); *(b)* the quality index 1 (QI1), which is an index for image degradation resulting from bulk motion, residual magnetization, incomplete spoiling and ghosting (22); and *(c)* the smoothness of voxels calculated as the full width at half maximum (FWHM) of the spatial distribution of image intensity values in voxel units (https://github.com/preprocessed-connectomes-project). This step was implemented on the whole sample and identified one severely affected dataset, which was marked for removal (Supplementary Figure S1A). To ensure that the remaining datasets did not contain further outliers, multivariate robust squared Mahalanobis distance outlier detection was additionally performed on vectors of median Δχ values extracted from the subcortical masks for each sample separately. This procedure identified four outlier datasets within the patient sample, which were marked for removal (Supplementary Figure S1B). Following quality control, group comparisons of magnitude image quality metrics (signal-to-noise ratio; SNR; contrast-to-noise ratio, CNR; foreground-to-background ratio, FBER; voxel smoothness; EFC; QI1) revealed no significant differences between patients and controls (Supplementary Table S1).

**1.5 Allen human brain atlas gene expression**

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**1.6 Iron-related gene sets**

**Supplementary Table S1: Iron homeostasis gene set (*N* = 12)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Gene name** | **Gene function** | **PC1** | **PC2** | **PC3** |
| FTH1 | ferritin, heavy polypeptide 1 | Encodes the heavy subunit of ferritin, the major intracellular iron storage protein in prokaryotes and eukaryotes. | 0.89 | −0.01 | 0.07 |
| FTL | ferritin, light polypeptide | Encodes the light subunit of the ferritin protein. | 0.87 | 0.07 | −0.06 |
| TF | transferrin | Transport iron from the intestine, reticuloendothelial system, and liver parenchymal cells to all proliferating cells in the body and brain. | 0.75 | 0.13 | −0.05 |
| IL6R | interleukin 6 receptor | A potent pleiotropic cytokine that regulates cell growth and differentiation and plays an important role in the immune response. | 0.71 | −0.13 | −0.05 |
| SLC40A1 | solute carrier family 40 (iron-regulated transporter), member 1 | A cell membrane protein that may be involved in iron export from duodenal epithelial cells. | 0.50 | 0.42 | −0.54 |
| IL6 | interleukin 6 (interferon, beta 2) | Encodes a cytokine that functions in inflammation and the maturation of B cells. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. | 0.13 | 0.48 | 0.27 |
| IL1A | interleukin 1, alpha | A pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis produced by monocytes and macrophages in response to cell injury, and thus induces apoptosis. | 0.05 | 0.65 | −0.43 |
| TNF | tumor necrosis factor | Encodes a multifunctional proinflammatory cytokine that is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism and coagulation. | 0.04 | 0.13 | 0.48 |
| HFE | hemochromatosis | Encodes a membrane protein that regules iron absorption by regulating the interaction of the transferrin receptor with transferrin. The iron storage disorder, hereditary haemochromatosis, is a recessive genetic disorder that results from defects in this gene. | −0.08 | 0.79 | 0.21 |
| TFR2 | transferrin receptor 2 | Encodes a single-pass type II membrane protein mediates cellular uptake of transferrin-bound iron. | −0.09 | 0.04 | 0.74 |
| HFE2 | hemochromatosis type 2 (juvenile) | Encodes a protein involved in iron metabolism by activating hepcidin or acting as a modulator of hepcidin expression. | −0.24 | 0.8 | 0.00 |
| IREB2 | iron-responsive element binding protein 2 | Encodes an RNA-binding protein that acts to regulate iron levels in the cells by regulating the translation and stability of mRNAs that affect iron homeostasis under conditions when iron is depleted. | −0.53 | 0.18 | 0.05 |

**Supplementary Table S2: Iron deficiency gene set (*N*=XX)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Gene name** | **Gene function** | **PC1** | **PC2** | **PC3** |
| MOG | Myelin oligodendrocyte glycoprotein | Encodes a membrane protein expressed on the oligodendrocyte cell surface and the outermost surface of myelin sheaths. Involved in completion and maintenance of the myelin sheath and in cell-cell communication. | 0.95 | −0.04 | 0.15 |
| TF | transferrin | Transport iron from the intestine, reticuloendothelial system, and liver parenchymal cells to all proliferating cells in the body and brain. | 0.95 | −0.19 | 0.04 |
| MOBP | Myelin-associated oligodendrocytic basic protein | Encodes a protein that plays a role in compacting or stabilizing the myelin sheath, possibly by binding the negatively charged acidic phospholipids of the cytoplasmic membrane. | 0.94 | −0.03 | 0.16 |
| MAL | Myelin and lymphocyte protein differentiation protein | Encodes an integral membrane protein localized to the endoplasmic reticulum of T-cells compact myelin of cells in the nervous system. Implicated in myelin biogenesis and/or function. | 0.93 | 0.03 | 0.21 |
| KLK6 | Kallikrein-related peptidase 6 | Member of the family of serine proteases implicated in carcinogenesis. The encoded protease may participate in the cleavage of amyloid precursor protein and alpha-synuclein, thus implicating it in Alzheimer’s and Parkinson’s disease. | 0.93 | −0.28 | 0.10 |
| CRYAB | Crystallin, alpha B | Encodes a moonlighting protein based on its ability to perform mechanistically distinct functions. Is a member of the small heat shock protein (HSP20) family and Also functions as an autokinase and participates in intracellular architecture. | 0.91 | −0.23 | −0.08 |
| CA2 | Carbonic anhydrase II | Encodes a protein that catalyzes reversible hydration of carbon dioxide. | 0.89 | 0.13 | 0.22 |
| APOD | Apolipoprotein D | Encodes a component of high density lipoprotein that is closely associated with the enzyme lecithin- an enzyme involved in lipoprotein metabolism. | 0.89 | −0.09 | 0.28 |
| STMN4 | Stathmin-like 4 | Encodes a protein involved in tubulin binding and cytoskeletal function. | 0.67 | 0.32 | −0.30 |
| CTSS | Cathepsin S | Encodes a lysosomal cysteine proteinase that may participate in the degradation of antigenic proteins to peptides for presentation on MHC class II molecules. | 0.38 | −0.2 | 0.51 |
| APOC1 | Apolipoprotein C-I | Encodes a protein primarily in the liver that is activated when monocytes differentiate into macrophages. Plays a role in high density lipoprotein (HDL) and very low density lipoprotein (VLDL) metabolism. | 0.28 | 0.04 | 0.71 |
| DCK | Deoxycytidine kinase | Encodes a protein required for the phosphorylation of several deoxyribonucleosides and their nucleoside analogs. Deficiency of DCK is associated with resistance to antiviral and anticancer chemotherapeutic agents. | 0.14 | 0.74 | −0.16 |
| LYZ | Lysozyme | Encodes human lysozyme, whose natural substrate is the bacterial cell wall peptidoglycan. Exhibits antibacterial activity against a number of bacterial species. | 0.09 | −0.53 | 0.41 |
| GSTM1 | Glutathione S-transferase mu 1 | Encodes a protein that enzymes functions in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione. | −0.16 | −0.21 | 0.67 |
| THRSP | thyroid hormone responsive protein | Encodes a nuclear protein important in the regulation of lipid metabolism. Is induced by thyroid hormone, carbohydrate intake, adipose tissue differentiation, and lactation, and is inhibited by glucagon and conjugated linoleic acid. | −0.19 | −0.62 | −0.47 |
| HOMER1 | Homer homolog 1 (Drosophila), neuronal immediate early gene | Encodes a member of the homer family of dendritic proteins. Associated with regulating group 1 metabotrophic glutamate receptor function. | −0.34 | 0.77 | −0.02 |
| RASGRP1 | RAS guanyl releasing protein 1 | Encodes a protein that functions as a diacylglycerol (DAG)-regulated nucleotide exchange factor specifically activating Ras through the exchange of bound GDP for GTP. It activates the Erk/MAP kinase cascade and regulates T-cells and B-cells development, homeostasis and differentiation. | −0.41 | 0.53 | −0.22 |

**Supplementary Table S3: Iron transport and uptake gene set (*N*=XX)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Gene name** | **Gene function** | **PC1** | **PC2** | **PC3** |
| FTL | ferritin, light polypeptide | Encodes the light subunit of the ferritin protein. | 0.82 | 0.14 | −0.13 |
| CYBRD1 | cytochrome b reductase 1 | A member of the cytochrome b(561) family that encodes an iron-regulatory protein that has ferric reductase activity and is believed to play a physiological role in dietary iron absorption. | 0.81 | −0.23 | 0.26 |
| FTH1 | ferritin, heavy polypeptide 1 | Encodes the heavy subunit of ferritin, the major intracellular iron storage protein in prokaryotes and eukaryotes. | 0.79 | 0.28 | −0.31 |
| HEPH | hephaestin | Encodes a member of the multicopper oxidase protein family. Involved in the transport of dietary iron from epithelial cells of the intestinal lumen into the circulatory system, and may be involved in copper transport and homeostasis. | 0.79 | −0.15 | −0.01 |
| ATP6V0E1 | ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1 |  | 0.78 | 0.13 | 0.40 |
| HMOX1 | heme oxygenase (decycling) 1 | Heme oxygenase occurs as the inducible isozyme HMOX1 and the isozyme constitutive HMOX2. The isozymes are essential in heme catabolism. | 0.78 | 0.10 | 0.18 |
| TF | transferrin | Transport iron from the intestine, reticuloendothelial system, and liver parenchymal cells to all proliferating cells in the body and brain. | 0.74 | 0.15 | 0.12 |
| ABCG2 | ATP-binding cassette, sub-family G (WHITE), member 2 | Encodes a membrane-associated protein included the superfamily of ATP-binding cassette (ABC) transporters. Involved in the transport of various molecules across extra- and intra-cellular membranes. | 0.72 | 0.05 | 0.41 |
| SLC40A1 | solute carrier family 40 (iron-regulated transporter), member 1 | Encodes a transmembrane protein that may be involved in iron export. | 0.69 | −0.09 | 0.41 |
| ATP6V0A4 | ATPase, H+ transporting, lysosomal V0 subunit a4 |  | 0.64 | −0.03 | 0.01 |
| TCIRG1 | T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 subunit A3 |  | 0.61 | 0.22 | 0.28 |
| ATP6V1E2 | ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2 |  | 0.55 | 0.14 | −0.25 |
| HMOX2 | heme oxygenase (decycling) 2 |  | 0.42 | 0.68 | −0.25 |
| SLC46A1 | solute carrier family 46 (folate transporter), member 1 | Encodes a transmembrane proton-coupled folate transporter protein. Facilitates the movement of folate and antifolate substrates across cell membranes and also functions as a heme transporter in duodenal enterocytes. | 0.42 | 0.01 | 0.49 |
| ATP6V1D | ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D |  | 0.28 | 0.59 | 0.30 |
| CP | ceruloplasmin (ferroxidase) | Encodes metalloprotein that is involved in the peroxidation of Fe(II)transferrin to Fe(III) transferrin, in addition to binding most of the copper in plasma. | 0.28 | 0.08 | 0.47 |
| ATP6V0B | ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b |  | 0.26 | 0.79 | 0.00 |
| ATP6V1E1 | ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1 |  | 0.25 | 0.62 | 0.16 |
| FLVCR1 | feline leukemia virus subgroup C cellular receptor 1 | Encodes a member of the major facilitator superfamily of transporter proteins. The protein is a heme transporter that may play a critical role in erythropoiesis by protecting developing erythroid cells from heme toxicity. | 0.24 | 0.22 | 0.72 |
| ATP6V1G1 | ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G1 |  | 0.18 | 0.72 | 0.31 |
| ATP6V0A2 | ATPase, H+ transporting, lysosomal V0 subunit a2 | Encodes a trans-membrane subunit of vacuolar ATPase (v-ATPase) which mediates acidification of intracellular of compartments of eukaryotic cells. Involved in zymogen activation, receptor-mediated endocytosis and synaptic vesicle proton gradient generation. The V0 domain consists of five different subunits and is involved in proton translocation. | 0.13 | 0.02 | 0.37 |
| ATP6V1C2 | ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2 |  | 0.10 | 0.24 | −0.59 |
| ATP6V1F | ATPase, H+ transporting, lysosomal 14kDa, V1 subunit F |  | 0.09 | 0.86 | −0.19 |
| ATP6V1B2 | ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2 |  | 0.08 | 0.78 | 0.06 |
| ATP6V0D2 | ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2 |  | 0.07 | −0.50 | −0.09 |
| ATP6V1G3 | ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G3 |  | 0.03 | −0.38 | −0.18 |
| TFRC | transferrin receptor 2 | Encodes a cell surface receptor necessary for cellular iron uptake by the process of receptor-mediated endocytosis. | 0.02 | 0.11 | −0.25 |
| ATP6V1H | ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H |  | −0.01 | 0.75 | −0.13 |
| ATP6V0C | ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c |  | −0.02 | 0.71 | −0.46 |
| MCOLN1 | mucolipin 1 | Member of the transient receptor potential (TRP) cation channel gene family. Encodes a transmembrane protein that localizes to intracellular vesicular membranes and is permeable to Ca2+, Fe2+, Na+, K+, and H+. | −0.02 | 0.57 | −0.49 |
| ATP6V0D1 | ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1 |  | −0.06 | 0.8 | −0.32 |
| ATP6V1C1 | ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1 |  | −0.12 | 0.71 | −0.35 |
| STEAP3 | STEAP family member 3, metalloreductase | Encodes a multipass membrane protein that functions as an iron transporter. | −0.13 | 0.02 | 0.52 |
| ATP6V1A | ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A | Encodes a cytosolic subunit of vacuolar ATPase (v-ATPase) which mediates acidification of intracellular of compartments of eukaryotic cells. Involved in zymogen activation, receptor-mediated endocytosis and synaptic vesicle proton gradient generation. The V1 domain consists of three A and three B subunits, two G subunits plus the C, D, E, F, and H subunits. The V1 domain contains the ATP catalytic site and is involved in ATP hydrolysis. | −0.19 | 0.89 | −0.15 |
| ATP6V1G2 | ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G2 |  | −0.41 | 0.35 | −0.18 |

**Supplementary Table S4: Iron storage gene set (*N*=XX)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Gene name** | **Gene function** | **PC1** | **PC2** | **PC3** |
| FTH1P20 | ferritin, heavy polypeptide 1 pseudogene 20 |  | 0.95 | 0.15 | 0.01 |
| FTH1 | ferritin, heavy polypeptide 1 | Encodes the heavy subunit of ferritin, the major intracellular iron storage protein in prokaryotes and eukaryotes. This gene has multiple pseudogenes. Several alternatively spliced transcript variants have been observed, but their biological validity has not been determined. | 0.95 | 0.04 | −0.01 |
| FTL | ferritin, light polypeptide | Encodes the light subunit of the ferritin protein. Has multiple pseudogenes | 0.89 | 0.01 | −0.06 |
| FTH1P14 | ferritin, heavy polypeptide 1 pseudogene 14 |  | 0.24 | 0.68 | 0.30 |
| FTMT | ferritin mitochondrial | Stores mitochondrial iron in a soluble and readily available form. | −0.01 | 0.90 | −0.02 |
| FTLP17 | ferritin, light polypeptide pseudogene 17 |  | −0.11 | 0.15 | 0.96 |
| FTH1P17 | ferritin, heavy polypeptide-like 17 | Encodes a ferritin heavy chain-like protein. Is primarily expressed in embryonic germ cells and may lack ferroxidase activity. |  |  |  |