# BRAIN IRON ACCUMULATION IN WILSON DISEASE: A PILOT 7T MR-HISTOPATHOLOGY CORRELATION STUDY

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**Target audience:** Imaging and clinical scientists, radiologists, pathologists, neurologists, ultra-high field experts

**Purpose:** Wilson disease (WD) is a genetic disorder caused by mutation of *ATP7B* leading to copper (Cu) metabolism disturbances and its gradual accumulation in liver and brain. Neurologic symptoms are presumably caused by Cu toxicity and associated with increased signal in basal ganglia, brainstem and thalami in magnetic resonance (MR) $T_2$weighted ($T_2$w) images reflecting tissue edema, cavitation, demyelination and/or gliosis[1]. During WD progression, decreased signal in globi pallidi (GP) and putamina in $T_2$w images suggestive of paramagnetic metal deposits may appear. It has been argued that these deposits could be formed by iron (Fe)[2] or certain $Cu^{2+}$ species with paramagnetic properties[3]. Our goal was to examine Fe content in post-mortem WD brains in relation to MR hyposignal.

**Methods:** Coronal brain slices from three neurological WD patients (all males, age at death 21, 22, 33 years, death due to complications of immobilization, fixation time 5, 15, 14 years) and one control subject (male, age at death 61 years, death due to aortal dissection, fixation time 1 year) were used. The brain slices were scanned while embedded in formalin using a 7.0T whole body MRI system (Magnetom, Siemens Healthcare, Erlangen, Germany) in conjunction with a 24 channel RF coil array dedicated for brain imaging (Nova Medical, Wilmington, MA, USA).. High spatial resolution images were acquired using $T_2$*w 3D gradient echo (GRE) dual echo (TR=50 ms, TE=6.1 and 19 ms, α=15°, resolution 0.15x0.15x0.5 $mm^3$, 7 averages, scan time 01:31 h) and MP-RAGE (TR=2300 ms, TE=5.9 ms, α=15°, TI= 700ms, resolution 0.1x0.1x0.5 $mm^3$, 15 averages, scan time 2:36 h) sequences. Additionally, $T_2$* mapping using a multi-echo GRE sequence (TR=500 ms, α=35°, 7 equidistant echoes ranging from 4.6ms to 47.2 ms) was performed. $T_2$* times were computed online using the scanner software and two regions of interest (ROI) were placed in the areas with most pronounced signal drop in GP and putamen. Blocks measuring approx. 3x2x0.5 cm containing basal ganglia were cut from the brain slices, embedded in paraffin, sliced with a microtome into 5 µm thick section and mounted on glass slides in the same orientation as the MR image acquisition. Hematoxylin-eosin staining for evaluation of general pathology and diaminobenzidine-enhanced Turnbull blue staining for iron were performed.

**Results:** Signal drop in GP and putamina of all WD brains suggestive of paramagnetic deposits was apparent in both, GRE and MP-RAGE (Fig1A) images. Mean $T_2$* relaxation times in the putaminal and GP ROIs were 5.1±(SD)0.5, 5.1±0.3, 5.2±0.3 ms and 5.8±1, 5.7±0.6 and 5.0±0.2 ms respectively in WD brains compared to 19.3±0.6 ms and 16.5±0.5 ms respectively in the control brain. Intensity of Turnbull Fe staining correlated fairly well with the low signal on MR on visual comparison (Fig1B,C). Normal pattern of Fe staining (iron present predominantly in myelinated bundles passing through putamen and GP) was disordered in all WD brains where GP and putamen showed profound diffuse Fe staining present in myelinated bundles, cellular elements, perivascular spaces and neuropil (Fig1D, F). The putamina of WD brain 1 and 2 displayed marked tissue rarefaction, cavitation and astrogliosis associated with profound signal loss on GRE and MP-RAGE images. This signal loss was apparently associated with the presence of heavily stained granular Fe deposits that were frequently associated with perivascular macrophages (Fig1E, G; arrows). The putamen and GP in WD brain 3 showed similar deposits, but with lower density, that were associated with a milder MR signal drop.

**Discussion:** The signal drop in GP and putamen of WD patients visible in GRE and MP-RAGE images is associated with the presence of granular deposits stained for iron by Turnbull staining. These deposits may be composed of aggregated ferritin or hemosiderin, compounds that are known for their marked paramagnetic properties[4]. Our results support the possibility that the $T_2$(*) hyposignal areas observed *in vivo* in basal ganglia of WD patients may be caused by deposited Fe rather than Cu compounds.

**Conclusion:** This is the first histopathological demonstration that neurological WD is associated with Fe accumulation in basal ganglia and these deposits can be visualized by MR imaging.

**References:** Sinha S et al. Neuroradiology. 2006;48(9):613-21. Skowronska M et al., Neurol Neurochir Pol. 2013;47(6):542-6. Fritzsch D et al., Investigative Radiology. 2014;49(5):299-306. Gossuin Y et al. NMR Biomed. 2007;20(8):749-56.

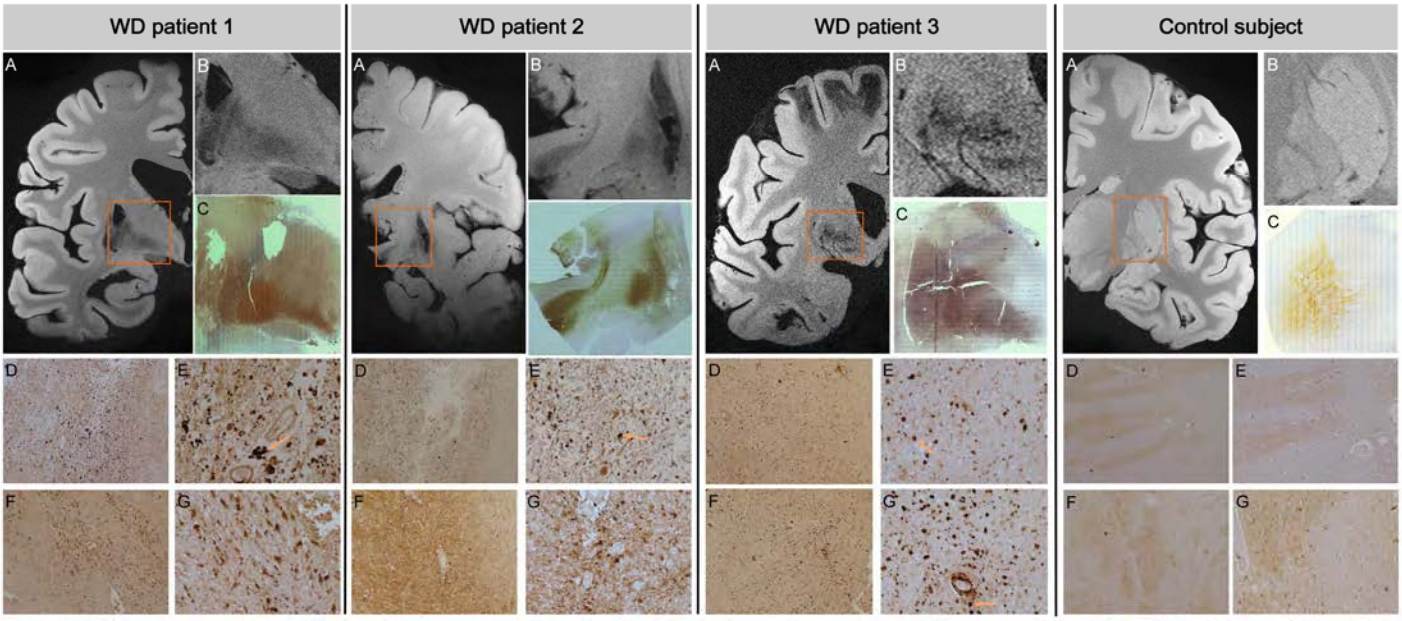


Fig.1: A) MP-RAGE overview image; B) detail of basal ganglia (red rectangle in A); C) overview of Turnbull stained sample; D) putamen (Turnbull) 10x, E) putamen (Turnbull) 40x; F) GP (Turnbull) 10x, G) GP (Turnbull) 40x