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Elevated Age-Related Cortical Iron, Ferritin and Amyloid Plaques in $APP_{swe}/PS1_{\Delta E9}$ Transgenic Mouse Model of Alzheimer's Disease

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Summary

Iron is very important element for functioning of the brain. Its concentration changes with aging the brain or during disease. The aim of our work was the histological examination of content of ferritin and free iron (unbound) in brain cortex in association with Aβ plaques from their earliest stages of accumulation in amyloid plaque forming APP/PS1 transgenic mice. Light microscopy revealed the onset of plaques formation at 8-monthage. Detectable traces of free iron and no ferritin were found around plaques at this age, while the rate of their accumulation in and around Aβ plaques was elevated at 13 months of age. Ferritin accumulated mainly on the edge of Aβ plaques, while the smaller amount of free iron was observed in the plaque-free tissue, as well as in and around Aβ plaques. We conclude that free iron and ferritin accumulation follows the amyloid plaques formation. Quantification of cortical iron and ferritin content can be an important marker in the diagnosis of Alzheimer's disease.

Key words

Iron • Ferritin • Cortex • Mouse brain • Light microscopy

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Introduction

Iron plays an important role in the normal brain cell metabolism and its concentration changes with age or diseases. Despite the importance of extracellular iron, it may be toxic in free form for the brain cells and disruption of its metabolism leads to neurodegenerative diseases (Meadowcroft *et al.* 2015). Normal iron metabolism is strictly controlled and excess iron is stored in ferritin protein (Dobson 2001). Ferritin (Ft) is composed of heavy (FtH) and light (FtL) subunits which have different physiological roles (Murray *et al.* 1987). The FtH subunit catalyses Fe^{2+} oxidation and is abundant in tissues requiring iron detoxification and cellular protection such as heart and brain, whereas the FtL subunit is responsible for nucleation and mineralization of nanocrystals (Quintana and Gutierrez 2010). The ratio of these two isoforms is altering by age, brain region and disease state (Connor *et al.* 1995). Typical sign of Alzheimer disease (AD) is the accumulation of amyloid-beta (Aβ) protein fibrils into extracellular plaques, intracellular neurofibrillary tangles and increased concentration of iron particles in diseased brains (Jarrett *et al.* 1993, El-Agnaf *et al.* 2000, Selkoe 2001, Everett *et al.* 2014, Scarpini *et al.* 2003). However, the causative relationship between iron accumulation and disease pathology has not been yet fully revealed. During the progression of AD, the amount of ferritin (mostly FtH) in cortex is increasing with age and observed in tissues with higher iron consumption (Connor *et al.* 1995).

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Accumulation of metals in amyloid plaques has been suggested to plays an important role in the AD pathology through generation of reactive oxygen species (Lee *et al.* 1999, Lovell *et al.* 1998, Miller *et al.* 2006, Huang *et al.* 1999). Yet, it is still not clear whether iron accumulation is the initial cause or a secondary consequence of the disease. Therefore, it is very important to understand how the distribution of iron and ferritin is changing over ages. It has also been suggested that Aβ plaque formation by themselves are not toxic without iron accumulation (Meadowcroft *et al.* 2009).

This study employed APP/PS1 transgenic mice, a common AD mouse model with amyloid plaques closely resembling those of human AD (Garcia-Alloza *et al.* 2006, Jankowsky *et al.* 2001). The aim of our work was to determine changes in the intracellular ferritin and iron content in cerebral cortex area caused by aging and neurodegeneration and to assess progress of iron aggregation around amyloid plaques in the APP/PS1 transgenic mice.

Material and Methods

Animals

In this experiment were used female mice carrying mouse/human amyloid precursor protein (APP) with the Swedish double mutation and human presenilin 1 (PS1) with deletion of exon 9 protein gene (APP/PS1 mice, Jankowsky *et al.*, 2004) and wild-type littermates (wt). Mice were kept in standard laboratory cages with stainless steel wire tops and standard bedding and in a controlled environment with 12 : 12 light-dark cycle with *ad libitum* access to water and feed. Experimental animals were sacrificed at different ages: 2.5 months, 8 months, 13 months and 24 months.

Collection of samples

Mice were anesthetized with an overdose of pentobarbital/chloralaldehyde mixture, and the brains were perfused with ice-cold saline through the left ventricle. The samples were fast-fixed in 4 % formaldehyde for 4 h and thereafter were dehydrated in 30 % saccharose overnight. Next day, the brain samples were cut into 35 μm coronal sections with a freezing microtome (Leica SM 2000F, Wetzlar, Germany). We selected sections at the level of 2.1 mm posterior to bregma for staining.

Histological staining and analysis

The sections were stained with Perl´s blue for iron (Meguro *et al.* 2007) and with Congo red for amyloid detection (Wilcock, Gordon, and Morgan 2006). Immunohistochemical staining was accomplished with specific antibodies (Thermofisher Scientific, Waltham, MA, USA) for both FtH and FtL chains detection. Rehydrated samples were incubated with anti-H or anti-L ferritin primary antibodies overnight. Next, HRP-conjugated secondary antibodies, followed by DAB (Sigma-Aldrich, Saint-Louis, Missouri, USA) were used to visualize both chains. For amyloid detection, Congo red staining was performed as described before (Wilcock *et al.* 2006). The samples were observed under optical microscope Zeiss Scope.A1 (Zeiss, Gottingen, Germany) with attached AxioCam MRc 5 camera (Zeiss, Gottingen, Germany) from 10 different places in the cerebral cortex area. Acquired data were processed with Fiji-ImageJ application (Schindelin *et al.* 2012).

Statistical analysis

Data were analyzed with GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). Normality of the data was tested with D´Agostino-Pearson test was performed followed by ANOVA with repeated measure (ANOVA-RM) test and afterwards we compare each age group with Unpaired t-test. The threshold for statistical significance was set to $p < 0.05$.

Results

Iron and ferritin content in the cortex

Iron distribution in the cortex changed across ages, with peak values observed at 8 months in APP/PS1 mice and at 13 months in wild-type mice (Fig.1). The Fe content decreased from the initial level in both genotypes by the age of 24 months, but the decline was more profound in APP/PS1 mice than WT mice. A significant genotype difference in Fe content was observed at 13 and 24 months, such that the values were lower in APP/PS1 mice (t test, p=** 13 months, p=*** 24 months). There is a general effect of genotype by age interaction (ANOVA-RM, p=**). Iron deposits in the cortex appeared of globular and sometimes irregular in shape with the diameter ranging from 1.5 to 10 μm.

FtH levels in wild-type mice cortex showed increasing tendencies with a peak at 13 months and a later a drop at 24 months. In contrast, in APP/PS1 mice FtH levels exhibited a continuous increase with age (ANOVA-RM, p=**). The FtH content in APP/PS1 was significantly elevated in comparison with wild-type mice at 8, 13 and 24 months of age (Fig. 1). Deposits of FtH in

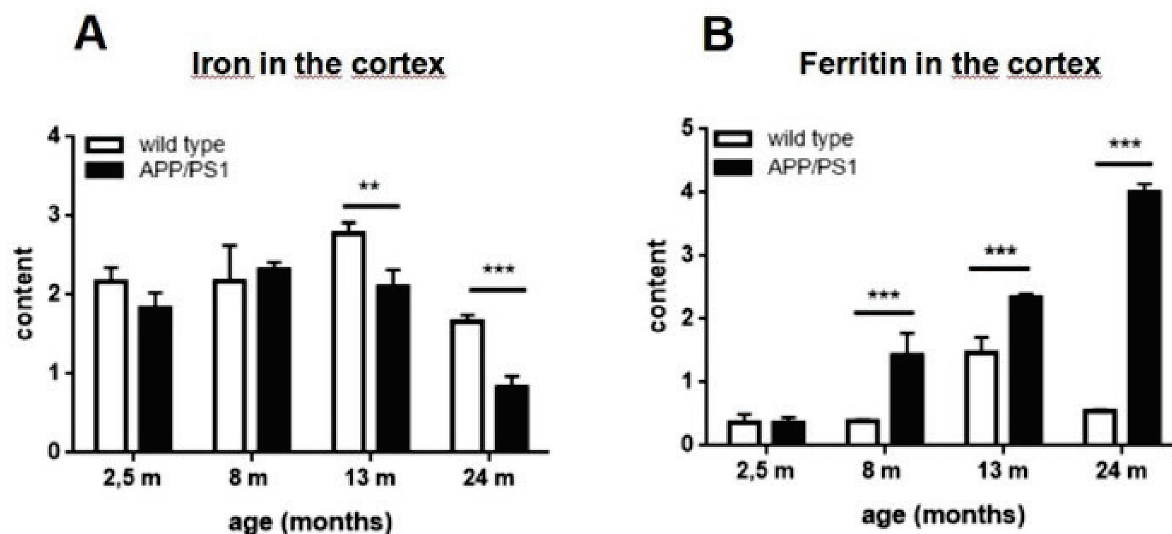

Fig. 1. (**A**) Iron content in the cortex of APP/PS1 mice (□) and control mice (■) over time. Black lines with asterisks indicate significant differences in Fe content (Mann-Whitney t-test, ** $p \leq 0.01$, *** $p \leq 0.001$, error bars represent SDs). (**B**) FtH content in the cortex of APP/PS1 mice (white bars) and control mice (dark bars) over time. Black lines with asterisks indicate significant difference in Fe content (Unpaired t-test, *** $p \leq 0.001$, error bars represent SDs).

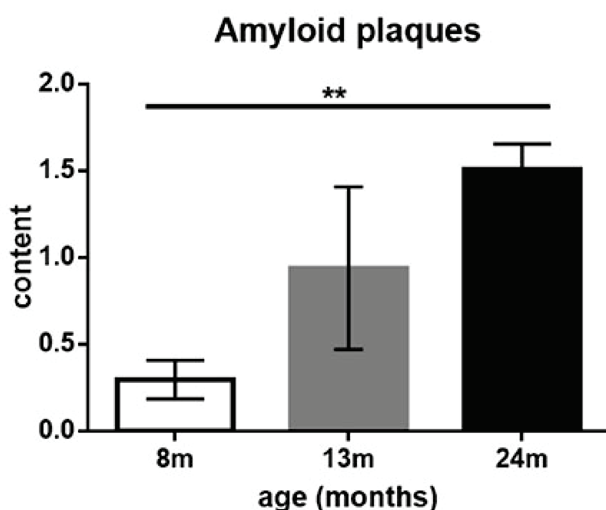

Fig. 2. Accumulation of amyloid plaques in APP/PS1 mice from the age of 8 months up to 24 months. Age-group means and SDs are shown.

the cortex were granular with the diameter ranging from 20 to 30 μm. Remarkably, the intensity of the staining was increased with age as well.

Amyloid distribution

Amyloid plaques were observed in the cortex of APP/PS1 animals from the age of 8 months on and increased steadily until 24 months of age (Fig. 2). The wild-type mice showed no staining positivity for human Aβ in the cortex.

Distribution of iron and FtH in the cortex of APP/PS1 mice showed differences between contents of FtH and iron. Eight months APP/PS1 mice had increased FtH and iron, but in 13 and 24 months we observed decrease of iron and persistent increase of FtH deposits. FtH accumulation occurred mainly on the edge of Aβ plaques as isolated depositions around plaques at 8 months old, with the number of deposits increased with age. The amount of iron around plaques and plaque free tissue was much less than that of FtH (Fig. 3).

Discussion

Iron is an essential element for proper cell functioning as well as a crucial catalyst for a number of chemical reactions. Its amount increases with age in cortex and other parts of human and mouse brain. Cerebral cortex in particular is affected by perturbation of iron distribution (Smith *et al.* 2010, van Duijn *et al.* 2017). AD is a typical example of neurodegenerative diseases where increased iron concentration is found in some parts of the brain (Connor *et al*. 1992, Jomova *et al.* 2010, Gong *et al*. 2019). Due to changed concentration during early stages of disease, iron is excellent tool for early diagnostic and these changes have high importance.

Our findings reveal that after an initial increase of cortical Fe content in APP/PS1 mice from 2.5 to 8 months of age, it is significantly lower than in wild-type mice at 13 and 24 months of age. This reduction in Fe content in older age in APP/PS1 mice can be explained by several mechanisms. The Perl's method we used stains mainly ferric iron (Fe^{3+}), albeit it stains some

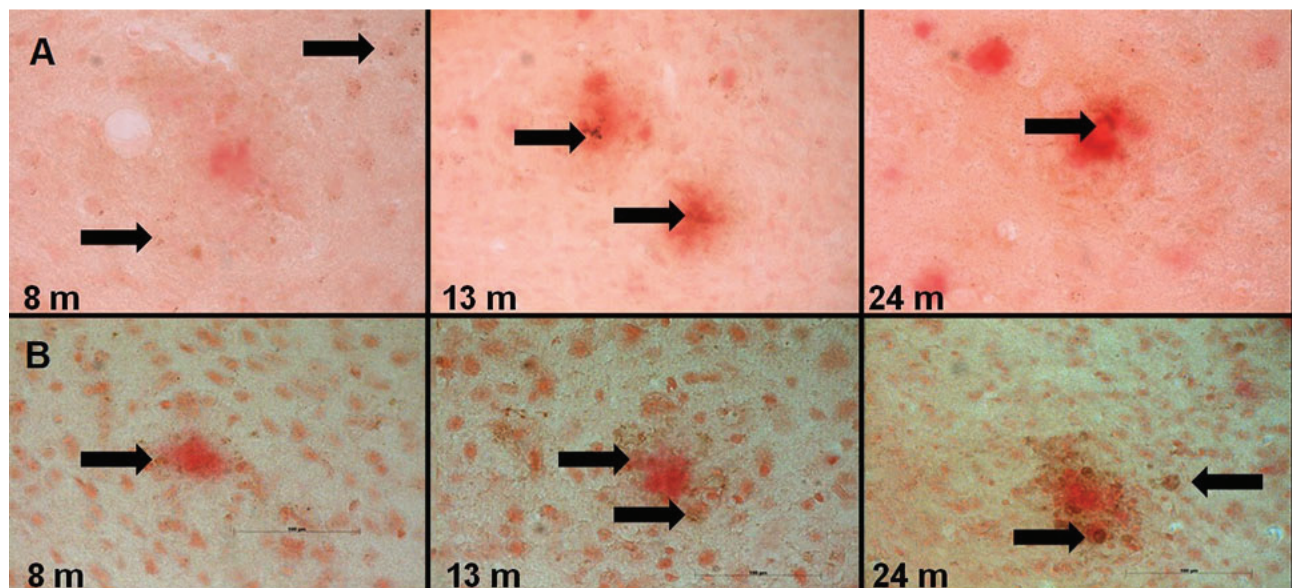

Fig. 3. FtH and Aβ plaque distribution in APP/PS1 mouse cortex across ages. It is evident that FtH accumulates around plaques (arrows).

ferrous iron (Fe^{2+}) as well (Meguro *et al.* 2007). The first mechanism involves Aβ formation and its capability to reduce Fe^{3+} ions to a divalent Fe^{2+} (Khan *et al.* 2006, Everett *et al.* 2014, Huang *et al.* 1999). Thereafter, increasing amounts of FtH can sequester increasing amount of Fe^{2+}, thereby protecting cells against iron-induced oxidative damage (Connor *et al.* 1994, Telfer and Brock 2002, Meadowcroft *et al.* 2015, Balejcikova *et al.* 2018). This explanation is supported by the fact that ferritin isolated from AD contains more iron ions than physiological ferritin (Bartzokis *et al.* 2004, Griffiths *et al.* 1999). Leskovjan *et al.* (2009) also observed decreased Fe content in cortex and explained that mature Aβ plaques may lose affinity for Fe ions. Hence, this effect stays behind the decrease of cortical Fe content. In addition, Perl's staining method cannot be able to stain the free iron inside Aβ plaques (Meadowcroft *et al.* 2009) and thus may decrease Fe content observed by histochemical analysis. The time course of cortical iron accumulation in APP/PS1 mice revealed maximum level of iron content between 10 and 12 months of age (Leskovjan *et al.* 2011, Xian-Hui *et al.* 2015). This age corresponds to appearance spatial memory impairments in this mouse model (Minkevicience *et al.* 2008). After the initial increase of Fe content in wild-type mice from 2.5 to 13 months of age, the Fe content tended to decrease. Our results are consistent with the results of Leskovjan *et al.* (2011) who observed decrease of Fe content in the mice cortex at 14 months.

FtH is the predominant type of ferritin in the brain and its content is increases with aging. Connor *et al.* (1995) suggest a cytoprotective antioxidant role of FtH through iron-sequestering capacity. Thus, elevated content of FtH in our samples could be a response to increased Fe content. However, the expression of FtH is regulated not only by iron but also for example by inflammatory cytokines what can point to robust neuroinflammatory reaction around amyloid plaques (Munro 1993). The relationship between FtH and iron should also be assessed from the view of histopathology. Aβ plaques show a striking co-localization with free iron and ferritin deposits (Smith *et al.* 1996, Lowell *et al.* 1998). Several authors suggest an interaction between Aβ and iron (Smith *et al.* 1997, Khan *et al.* 2006) resulting in the generation of reactive oxygen species. Telling *et al.* (2017) found formation of an iron-Aβ complex, but in contrast to our results, they observed that iron distribution matches the distribution of Aβ plaques. It is still not understood whether iron seeds Aβ plaque formation or vice versa, i.e. plaques induce iron deposition. Our analysis of the time course Aβ plaque formation shows that this process may not dependent on iron and ferritin accumulation. We found that the onset of Aβ plaques formation at 8 months old contents amyloid plaques with and without iron and iron increases with age. With respect to our findings, we suggest that iron/ferritin is consequence of Aβ formation and it is integrated later within plaques.

Although APP/PS1 mouse is a suitable model for studying AD-related amyloid plaque formation, it is

necessary to be very careful when extrapolating results of iron deposition in this mouse model to humans. Meadowcroft *et al.* (2015) observed the presence of diffuse iron in cortical Aβ plaques of APP/PS1 mice. They used histological staining methods likewise was used in our experiment or as used Van Duijn *et al.* (2017). Detection of the iron is possible by usage of different methods. Most of them are invasive and they are performed on tissue *ex vivo.* Bourassa *et al.* (2014) and Leskovjan *et al.* (2011) used synchrotron X-ray fluorescence microscopy (XFM), Xian-Hui et al (2015) used graphite furnace atomic absorption spectrometry (GFAAS) and also histology. SQUID (Superconducting Quantum Interference Device) magnetometry used Bulk *et al.* (2018) to detection of iron oxides and Kopani *et al.* (2015) which used also Mössbauer spectrometry (MS). Although these methods are suitable for iron changes experiments, for diagnostic are more appropriate non-invasive methods. Magnetic resonance (MRI) is able to detect iron in the brain *in vivo* or others new imaging methods (Fujiwara *et al.* 2014, House *et al.* 2007). Also, the new imaging methods based on magnetic properties of iron particles for visualizing and quantifying Aβ plaques and iron/ferritin in animal model and in the AD are important for preclinical and clinical research and early diagnosis of AD (Tadic *et al*. 2014).

Amyloid plaque formation has an effect on the level of iron in brain. We found the presence of amyloid plaques in the cortex of 8-month-old APP/PS1 mice without traces of free iron, while ferritin was found around plaques at this time. The accumulation of free iron and ferritin started around 13 months of age. We observed higher amount of ferritin accumulation than free iron deposits. Ferritin accumulated mainly on the edge of Aβ plaques. Furthermore, we did not find any correlation between the free iron distribution and the distribution of Aβ plaques. We conclude that H ferritin accumulation follows the amyloid plaques formation. Quantification of cortex iron and ferritin content can be an important marker for diagnosis of AD.

Conflict of Interest

There is no conflict of interest.

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References

BELEJCIKOVA L, SIPOSOVA K, KOPCANSKY P, SAFARIK I: Fe(II) formation after interaction of the amyloid ß-peptide with iron-storage protein ferritin. *J Biol Phys* **44**: 237-243, 2018.

BARTZOKIS G, TISHLER TA, SHIN IS, LU PH, CUMMINGS JL: Brain ferritin iron as a risk factor for age at onset in neurodegenerative diseases. *Ann NY Acad Sci* **1012**: 224-236, 2004.

BOURASSA MW, LESKOVJAN AC, TAPPERO RV, FARQUHAR ER, COLTON A, VAN NOSTRAND WE, MILLER LM: Elevated Copper in the Amyloid Plaques and Iron in the Cortex Are Observed in Mouse Models of Alzheimer's Disease That Exhibit Neurodegeneration. *Biomed Spectrosc Imaging* **2**: 129-39, 2014.

BULK M, VAN DER WEERD L, BREIMER W, LEBEDEV N, WEBB A, GOEMAN JJ, WARN RJ, HUBER M, OOSTERKAMP TH, BOSSONI L: Quantitative Comparison of Different Iron Forms in the Temporal Cortex of Alzheimer Patients and Control Subjects. *Scientific Reports* **8**: 1-11, 2018.

CONNOR JR, SNYDER BS, BEARD JL, FINE RE, MUFSON EJ: Regional distribution of iron and iron-regulatory proteins in the brain in aging and Alzheimer's disease. *J Neurosci Res* **31**: 327-335, 1992.

CONNOR JR, BOESHORE KL, BENKOVIC SA, MENZIES SL: Isoformsof ferritin have a specific cellular distribution in the brain. *J Neurosci Res* **37**: 461-465, 1994.

CONNOR JR, SNYDER BS, AROSIO P, LOEFFLER DA, LEWITT P: A quantitative analysis of isoferritins in select regions of aged, parkinsonian, and alzheimer's diseased brains. *J Neurochem* **65**: 717-724, 1995.

DOBSON J: Nanoscale biogenic iron oxides and neurodegenerative disease. *FEBS Lett* **496**: 1-5, 2001.

EL-AGNAF OMA, MAHIL DS, PATEL BP, AUSTEN BM: Oligomerization and toxicity of *β*-amyloid-42 implicated in Alzheimer's disease. *Biochem Biophys Res Commun* **273**: 1003-1007, 2000.

EVERETT J, CESPEDES E, SHELFORD LR, EXLEY C, COLLINGWOOD JF, DOBSON J, VAN DER LAAN G, JENKINS CA, ARENHOLZ E, TELLING ND: Evidence of redox-active iron formation following aggregation of ferrihydrite and the Alzheimer's disease peptide *β*-amyloid. *Inorg Chem* **53**: 2803-2809, 2014.

FUJIWARA S, UHRIG L, AMADON A, JARRAYA B, LE BIHAN D: Quantification of Iron in the Non-Human Primate Brain with Diffusion-Weighted Magnetic Resonance Imaging. *NeuroImage* **102**: 789-797, 2014.

GARCIA-ALLOZA M, ROBBINS EM, ZHANG-NUNES SX, PURCELL SM, BETENSKY RA, RAJU S, PRADA C, GREENBERG SM, BACSKAI BJ, FROSCH MP: Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. *Neurobiol Dis* **24**: 516-524, 2006.

GONG NJ, DIBB R, BULK M, VAN DER WEERD L, LIU CL: Imaging beta amyloid aggregation and iron accumulation in Alzheimer's disease using quantitative susceptibility mapping MRI. *Neuroimage* **191**: 176-185, 2019.

GRIFFITHS PD, DOBSON BR, JONES GR, CLARK DT: Iron in the basal ganglia in Parkinson's disease. An in vitro study using extended X-ray absorption fine structure and cryo-electron microscopy. *Brain* **122**: 667-673, 1999.

HOUSE MJ, ST PIERRE TG, KOWDLEY KV, MONTINE T, CONNOR J, BEARD J, BERGER J, SIDDAIAH N, SHANKLAND E, JIN LW: Correlation of proton transverse relaxation rates (R2) with iron concentrations in postmortem brain tissue from Alzheimer's disease patients. *Magnetic Resonance in Medicine* **57**: 172-180, 2007.

HUANG XD, ATWOOD CS, HARTSHORN MA, MULTHAUP G, GOLDSTEIN LE, SCARPA RC, CUAJUNGCO MP, GRAY DN, LIM J, MOIR RD, TANZI RE, BUSH AI: The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. *Biochem* **38**: 7609 - 7616, 1999.

JANKOWSKY JL, FADALE DJ, ANDERSON J, XU GM, GONZALES V, JENKINS NA, COPELAND NG, LEE MK, YOUNKIN LH, WAGNER SL, YOUNKIN SG, BORCHELT DR. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet* **13**: 159-170, 2004.

JARRETT JT, BERGER EP, LANSBURY PT: The carboxy terminus of the *β* amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochem* **32**: 4693-4697, 1993.

JOMOVA K, VONDRAKOVA D, LAWSON M, VALKO M: Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* **345**: 91-104, 2010.

KHAN A, DOBSON JP, EXLEY C: Redox cycling of iron by Abeta42. *Free Radic Biol Med* **40**: 557-569, 2006.

KOPANI M, MIGLIERINI M, LANCOK A, DEKAN J, CAPLOVICOVA J, JAKUBOVSKY M, BOCA R, MRAZOVA H: Iron oxides in human spleen. *Biometals* 28: 913-928. 2015.

LEE JY, MOOK-JUNG I, KOH JY: Histochemically reactive zinc in plaques of the Swedish mutant β-amyloid precursor protein transgenic mice. *J Neurosci* **19**: RC10, 1999.

LESKOVJAN AC, LANZIROTTI A, MILLER LM: Amyloid plaques in PSAPP mice bind less metal than plaques in human Alzheimer's disease. *Neuroimage* **47**: 1215-1220, 2009.

LESKOVJAN AC, KRETLOW A, LANZIROTTI A, BARREA R, VOGT S, MILLER LM: Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease. *Neuroimage* **55**: 32-38, 2011.

LOWELL MA, ROBERTSON JD, TEESDALE WJ, CAMPBELL JL, MARKRSBERY WR: Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci* **158**: 47-52, 1998.

MEADOWCROFT MD, CONNOR JR, SMITH MB, YANG QX: MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. *J Magn Reson Imaging* **29**: 997-1007, 2009.

MEADOWCROFT MD, CONNOR JR, YANG QX: Cortical iron regulation and inflammantory response in Alzheimer's disease and $APP_{SWE}/PS1_{\Delta E9}$ mice: a histological perspective. *Front Neurosci* **9**: 255, 2015.

MEGURO R, ASANO Y, ODAGIRI S, LI C, IWATSUKI H, SHOUMURA K: Nonheme iron histochemistry for light and electron microscopy: a historical, theoretical and technical review. *Arch Histol Cytol* **70**: 1-19, 2007.

MILLER LM, WANG Q, TELIVALA TP, SMITH RJ, LANZIROTTI A, MIKLOSSY J: Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with β-amyloid deposits in Alzheimer's disease. *J Struct Biol* **155**: 30-37, 2006.

MINKEVICIENE R, IHALAINEN J, MALM T, MATILAINEN O, KEKSA-GOLDSTEINE V, GOLDSTEINS G, IIVONEN H, LEGUIT N, GLENNON J, KOISTINAHO J, BANERJEE P, TANILA H. Age-related decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice. *J Neurochem* **105**: 584-594, 2008.

MUNRO HN: The ferritin genes: their response to iron status. *Nutr Rev* **51**: 65-73, 1993.

MURRAY MT, WHITE K, MUNRO HN: Conservation of ferritin heavy subunit gene structure: implications for the regulation of ferritin gene expression. *PNAS* **84**: 7438-7442, 1987.

QUINTANA C, GUTIERREZ L: Could a dysfunction of ferritin be a determinant factor in the aetiology of some neurodegenerative diseases? *Biochim Biophys Acta* **1800**: 770-782, 2010.

SELKOE DJ: Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81**: 741-766, 2001.

SCARPINI E, SCHELTENS P, FELDMAN H: Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol* **2**: 539-547, 2003.

SCHINDELIN J, ARGANDA-CARRERAS I, FRISE E, KAYNIG V, LONGAIR M, PIETZSCH T, PREIBISCH S, RUEDEN C, SAALFELD S, SCHMID B, TINEVEZ JY, WHITE DJ, HARTENSTEIN V, ELICEIRI K, TOMANCAK P, CARDONA A: Fiji: an open-source platform for biological-image analysis. *Nat Methods **9**:* 676-682, 2012.

SMITH MA, HARRIS PLR, SAYRE LM, PERRY G: Is Alzheimer's disease of oxidative stress? *Alzheimers Dis Rev* **1**: 63-67, 1996

SMITH MA, SAYRE LM, PERRY G: Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *PNAS* **94**: 9866-9868, 1997.

SMITH MA, ZHU X, TABATON M, LIU G, MCKEEL JR DW, COHEN ML, WANG X, SIEDLAK SL, DWYER BE, HAYASHI T, NAKAMURA M, NUNOMURA A, PERRY G: Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. *J Alzheimers Dis* **19**: 363-372, 2010.

TADIC M, PANJAN M, DAMNJANOVIC V, MILOSEVIC I: Magnetic properties of hematite (α-Fe2O3) nanoparticles prepared by hydrothermal synthesis method. *Appl Surf Sci* **320**: 183-187, 2014.

TELFER JF, BROCK JH: Expression of ferritin, transferrin receptor, andnon-specific resistance associated macrophage proteins 1 and 2 (Nramp1 and Nramp2) in the human rheumatoid synovium. *Ann Rheu Dis* **61**: 741-744, 2002.

TELLING ND, EVERETT J, COLLINGWOOD JF, DOBSON J, VAN DER LAAN G, GALLAGHER JJ, WANG J, HITCHCOCK AP: Iron biochemistry is correlated with amyloid plaque morphology in an established mouse model of Alzheimer's disease. *Cell Chem Biol* **24**: 1205-1215, 2017.

VAN DUIJN S, BULK M, VAN DUINEN SG, NABUURS RJA, VAN BUCHEM MA, VAN DER WEERD L, NATTE R: Cortical iron reflects severity of Alzheimer's disease. *J Alzheimers Dis* **60**: 1533-1546, 2017.

WILCOCK DM, GORDON MN, MORGAN D: Quantification of cerebral amyloid angiopathy and parenchymal amyloid plaques with Congo red histochemical stain. *Nat Pro* **1**: 1591-1595, 2006.

XIAN-HUI D, WEI-JUAN G, TIE-MEI S, HONG-LIN X, JIANG-TAO B, JING-YI Z, XI-QING C: Age-related changes of brain iron load changes in the frontal cortex in APPswe/PS1E9 transgenic mouse model of Alzheimer's disease. *J Trace Elements Med Biol* **30**: 118-123, 2015.