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MORPHOLOGICAL AND BIOCHEMICAL SIGNS OF AGE-RELATED NEURODEGENERATIVE CHANGES IN KLOTHO MUTANT MICE

M. SHIOZAKI,[a,c] K. YOSHIMURA,[b] M. SHIBATA,[b] M. KOIKE,[b] N. MATSUURA,[c] Y. UCHIYAMA[b] AND T. GOTOW[a]*

[a]*Laboratory of Cell Biology, College of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo 665-0006, Japan*

[b]*Department of Cell Biology and Neurosciences, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*

[c]*Department of Molecular Pathology, Osaka University Graduate School of Medicine and Health Science, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan*

Abstract—Klotho mutant mice, defective in the klotho gene, develop multiple age-related disorders with very short lifespans. Introduction of the exogenous klotho gene into these mutant mice leads to an improvement in their phenotypes, while overexpression of this gene in wild-type mice significantly extends their lifespan. These observations suggest that the klotho gene/protein has an anti-aging function. Since there have been only a few reports with some disagreement about results on the CNS of the mutant mice, we tried to clarify whether the CNS neurons generate aging-like features, even in premature stages, using biochemical and morphological approaches. Results obtained from the mutant mice, when compared with wild-type mice, were as follows. Neurofilaments (NFs) were increased significantly in axons, with the subunit proteins showing a significant enhancement in phosphorylation or expression of NF-H or NF-L, respectively. Microtubules in Purkinje cell dendrites were closer to each other, and in the CNS tissue tubulin was unaltered, but microtubule-associated protein (MAP) 2 was significantly reduced in expression. Neuronal cellular organelles were morphologically disordered. Lysosomes, cathepsin D and light chain 3 of MAP1A/B (LC3) were augmented with the appearance of putative autophagy-related structures. Antiapoptotic Bcl-xL and proapoptotic Bax were reduced and enhanced, respectively, and mitogen-activated protein kinase was reduced. Synapse-related proteins and structures were decreased. Neuronal degeneration was evident in hippocampal pyramidal cells, and possibly in Purkinje cells. Astrocytic glial filaments and glial fibrillary acidic protein were increased in density and expression, respectively. Together, the CNS neuronal alterations in klotho mutant mice were quite similar to those found in aged animals, including even premature death, so this mouse should be a more appropriate animal model for CNS aging than those previously reported. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

*Corresponding author. Tel: +81-797-87-8140; fax: +81-797-87-5666.
E-mail address: gotow@koshien.ac.jp (T. Gotow).

Abbreviations: CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; LC3, light chain 3 of microtubule-associated protein 1A and B; MAP, microtubule-associated protein; MAPK, mitogen-activated protein kinase; NF, neurofilament; PBS, phosphate-buffered saline; SAMP, senescence-accelerated mouse prone; TBS, Tris-buffered saline.

Key words: cytoskeleton, lysosome, cell death–associated protein, synapse, astrocyte.

The klotho mutant mouse, which is defective in the klotho gene, is considered to be a novel animal model for accelerated human aging, because it displays an extremely short lifespan (around 8 weeks) and even at the age of 4–5 weeks is prone to multiple syndromes and deteriorations related to aging in humans: arteriosclerosis, osteoporosis, skin atrophy, infertility, thymic atrophy and pulmonary emphysema (Kuro-o et al., 1997). Further evidence that the klotho gene is responsible for aging comes from two phenomena: the introduction of a normal klotho gene into the mutant mice stops their age-dependent deterioration (Kuro-o et al., 1997), and overexpression of this gene in normal wild-type mice extends their lifespans (Kurosu et al., 2005). Klotho mutant mice, however, do not reveal some phenotypes usually seen in aged humans, such as brain atrophy with the deposition of amyloid or senile plaque (Kuro-o et al., 1997; Nagai et al., 2003; Anamizu et al., 2005; Nixon et al., 2005). Since neurodegenerative changes with senile plaque/amyloid deposits are not visible in any aged rodents, it is unknown whether the CNS of klotho mutant mice shows any age-related alterations.

We noted the observation by Kuro-o et al. (1997) of hypokinesis and gait abnormalities, and the analysis by Nagai et al. (2003) of cognitive impairment in klotho mutant mice. An ambulatory disorder, especially of the posterior limbs, is readily apparent in these mice, suggesting a disorder of motor neurons. Although the loss or degeneration of Purkinje cells was suggested by Kuro-o et al. (1997), no morphological evidence has been published. The klotho gene in humans, which shares 86% of its encoded protein with that of the mouse (Kuro-o et al., 1997), showed functional variants associated with altered or reduced lifespan and risk for coronary artery disease or stroke (Arking et al., 2002, 2003, 2005). The klotho gene variation may also be associated with the decline of age-related cognitive ability (Deary et al., 2005), supporting the idea that this klotho gene is responsible for age-dependent neuronal degeneration in humans.

Because of cognitive impairment in klotho mutant mice (Nagai et al., 2003), the hippocampus of these animals has been analyzed by two groups. Biochemically, Nagai et al. (2003) showed that the anti-death gene/protein, Bcl-2 or Bcl-xL, was downregulated while the pro-death molecule, Bax, was upregulated in these mutant mice. Immunocytochemically, Li et al. (2004) indicated that synaptic structures and synaptophysin were reduced in number and expression, respectively, in the CA3 region of these ani-

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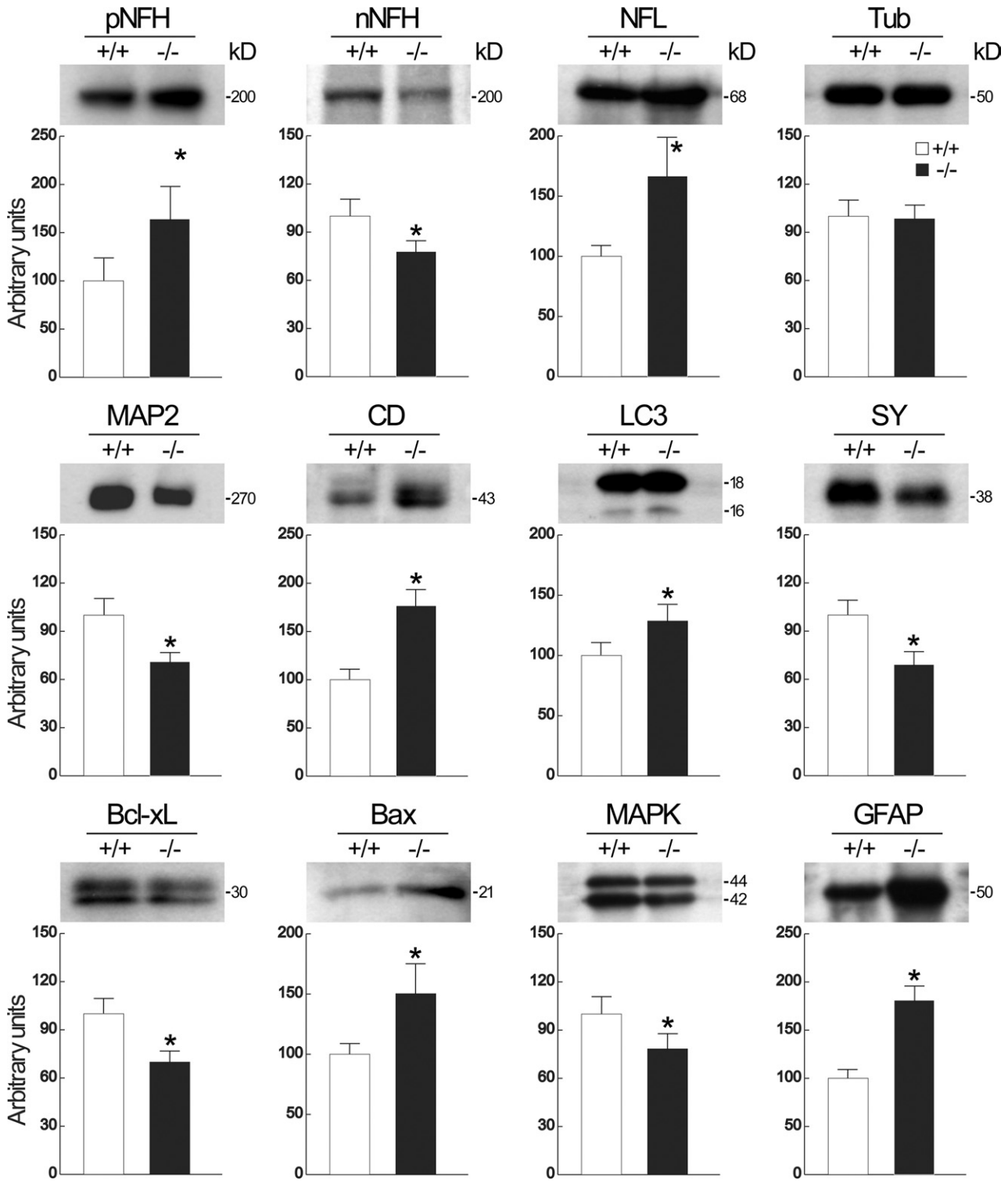

Fig. 1. Immunoblots of CNS tissue extracts from 7-week-old, wild-type (+/+) and homozygous mutant (−/−) mice. Both mouse CNS tissues were homogenized and immunoblotted with antibodies that recognize each specific protein. Each is represented above by blotted bands in the upper panels. Intensities of the respective bands from wild-type and mutant mice were represented as graphs and statistically evaluated. Quantitative data were shown as mean±S.D. The band intensity from the wild-type mice was expressed as mean value 100 in arbitrary units. Seven to 17 samples from four wild-type and four mutant mice were used for the quantification. Compared with wild-type mice, in mutant mice expressions for phosphorylated NF-H (pNFH), NF-L (NFL), cathepsin D (CD), LC3, Bax and GFAP were significantly higher (* $P<0.001$). However, other proteins, i.e. nonphosphorylated NF-H (nNF-H), MAP2, synaptophysin (SY), Bcl-xL and MAPK were significantly less expressed (* $P<0.001$) in the mutant mice than in the wild-type mice. No significant difference in α-tubulin (Tub) expression was found.