

## Copper Biology in Health and Disease

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# The accumulation of copper in the brain of Down syndrome promotes oxidative stress: possible mechanism underlying cognitive impairment

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Individuals with Down syndrome (DS), which is caused by triplication of human chromosome 21 (Hsa21), show numerous characteristic symptoms, such as intellectual disability, an impaired cognitive function, and accelerated aging-like phenotypes. Enhanced oxidative stress is assumed to be implicated as a mechanism underlying many of these symptoms of DS. Some genes coded in Hsa21, such as *App*, *Sod1*, and *Ets2*, are suggested as being involved in the exacerbation of oxidative stress. In addition, enhanced oxidative stress has been recently shown to be caused by dyshomeostasis of the redox-active bio-metal copper in the brain of a mouse model of DS. This review aims to summarize the current knowledge on enhanced oxidative stress in DS and suggest a possible molecular mechanism underlying the cognitive impairment of DS mediated by enhanced oxidative stress.

**Key Words:** Down syndrome, oxidative stress, copper, cognitive impairment

Down syndrome (DS), caused by triplication of human chromosome 21 (Hsa21), is the most frequent aneuploidy, occurring in approximately 1 in 700 live births.<sup>(1)</sup> DS is characterized by developmental retardation, intellectual disability, craniofacial abnormalities, and hypotonia.<sup>(2)</sup> Most individuals with DS exhibit mild to moderate learning disability. Overexpression of some Hsa21 genes, such as amyloid precursor protein (APP),<sup>(3)</sup> regulator of calcineurin-1 (RCAN1),<sup>(4,5)</sup> dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A),<sup>(6)</sup> synaptojanin 1,<sup>(7)</sup> and single-minded homolog 2,<sup>(8)</sup> has been suggested to be associated with learning and memory defects in mice. Therefore, gene-dosage imbalance of Hsa21 genes due to triplication is considered a major cause of learning anomalies in individuals with DS.<sup>(9,10)</sup>

The Hsa21 genes suggested to be involved in cognitive impairment have been identified through investigations using transgenic mice, and the candidate genes have been shown to be expressed at extremely high levels. However, the contribution of each gene to the cognitive impairment in DS is believed to be limited, suggesting that triplication of several Hsa21 genes may participate in cognitive impairment cooperatively.

It is widely accepted that enhanced oxidative stress (OS) can

lead to cognitive impairment. Indeed, increased levels of lipid peroxidation products have been detected in models of vascular dementia and Alzheimer's disease (AD), which exhibit cognitive impairment.<sup>(11)</sup> Enhancement of OS is caused by the increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and/or a decreased ROS/RNS scavenging ability. The overproduction of ROS has been shown in neuronal cells obtained from DS fetuses.<sup>(12)</sup> Erythrocytes from DS individuals show significantly higher levels of malondialdehyde (MDA), a typical product of lipid peroxidation, than controls.<sup>(13)</sup> Furthermore, mitochondrial dysfunction and the overproduction of ROS are detected in fibroblasts from human fetuses with DS.<sup>(14)</sup> In addition, evidence of enhanced OS in mouse models of DS has been accumulated.<sup>(15–17)</sup>

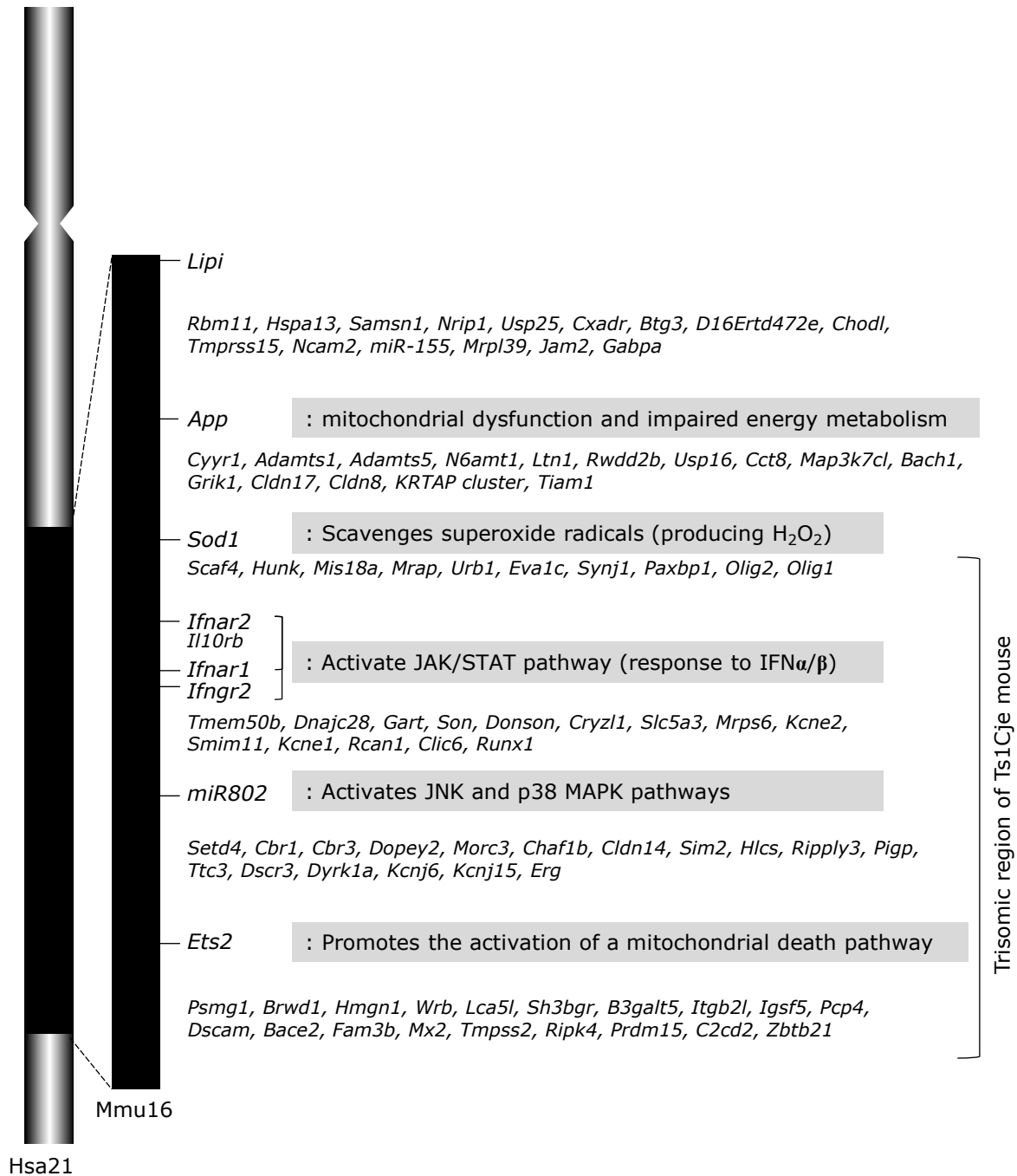
In this review, we will discuss the molecular mechanisms underlying the enhanced OS in DS models and the possibility of cognitive impairment in DS caused by enhanced OS.

### Hsa21 Genes Associating with Enhanced OS

Several genes coded in Hsa21 are suggested to be associated with enhanced OS in DS (Fig. 1). The increased expression of superoxide dismutase 1 (SOD1) is one particularly promising candidate potentially involved in enhanced OS. The overexpression of SOD1 results in the overproduction of H<sub>2</sub>O<sub>2</sub>. Normally, H<sub>2</sub>O<sub>2</sub> is catabolized into H<sub>2</sub>O by catalase (CAT) and glutathione peroxidase (GPX), but the copy number of these genes are normal. Thus, increasing the expression of SOD1 without increasing the expression of CAT and GPX may lead to the accumulation of hydrogen peroxide in DS. Supporting this hypothesis, an increased ratio of SOD1 to [CAT + GPX] and enhanced lipid peroxidation have been shown in fibroblasts derived from fetuses with DS and erythrocytes from children with DS.<sup>(18,19)</sup>

The *APP* gene coded in Hsa21 is also suggested to be involved in mitochondrial dysfunction in DS. The increased expression of APP in mitochondria results in the progressive accumulation of transmembrane-arrested APP and causes mitochondrial dysfunction and impaired energy metabolism, suggesting that

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**Fig. 1.** Genes coded in Mmu16 syntenic to Hsa21 and annotated genes possible involved in enhanced OS. The region of Mmu16 from *Lipi* to *Zbtb21* is syntenic to a large portion of Hsa21. Annotated genes in the Mmu16 syntenic to Hsa21, which are suggested to be involved in enhanced oxidative stress, are presented in large letters.

the overexpression of APP may enhance OS via mitochondrial dysfunction in DS.<sup>(20)</sup>

The involvement of Hsa21 genes other than *App* and *Sod1* in enhanced OS has also been suggested. Ts1Cje mice, a model of DS carrying an extra copy of mouse chromosome 16 (Mmu16), which is orthologous to Hsa21, shows enhanced OS in the brain, although the trisomic region of the Ts1Cje mouse does not include the *Sod1* gene.<sup>(15,17)</sup> In the trisomic region of Ts1Cje mice, the *Rcan1*, *Ifnar2*, *Ifnar1*, *Ifngr2*, and *Ets2* genes have shown potential association with enhanced OS in DS. RCAN1-deficient neurons display an increased resistance to damage by H<sub>2</sub>O<sub>2</sub>,

suggesting that RCAN1 increases neuronal susceptibility to OS.<sup>(21)</sup> Neurons accumulating chronically RCAN1 longer isoform 1, which is highly expressed in the central nervous system with DS, promote OS-induced apoptosis with caspase-3 activation.<sup>(22)</sup> Increasing the copy number of interferon receptor genes in Mmu16, *Ifnar2*, *Ifnar1*, and *Ifngr2* is assumed to result in the overactivation of Jak-Stat signaling in response to type I interferon.<sup>(23)</sup> It has been shown that type I interferon signaling is activated in the aged brain and correlates with increased OS.<sup>(24)</sup> The overexpression of ETS2 in human cortical neurons with DS is suggested to promote neuronal apoptosis through mitochon-

drial dysfunction.<sup>(25)</sup> In addition to the protein-coding gene in Mmu16, miR-802 is also coded in the trisomic region of Ts1Cje mice. miR-802 is suggested to activate the JNK and p38 MAPK pathways and induce hepatic OS.<sup>(26)</sup> Although the triplication of such protein-coding genes and this miRNA may cooperatively enhance OS in DS, the mechanisms underlying the enhanced OS in DS remain unclear.

Enhanced OS is suggested to associate with cognitive impairment in DS. Indeed, experiments using mouse models of DS have demonstrated that reducing OS via vitamin E treatment improved the cognitive impairment in Ts65Dn mice carrying a trisomic region that was longer than that in Ts1Cje mice.<sup>(16,27)</sup> Unfortunately, antioxidants have shown no efficacy on the impaired cognitive function of adult humans with DS;<sup>(28,29)</sup> however, the involvement of several issues, such as low transitivity into the brain, has been considered. Further clinical trials may demonstrate the effectiveness of supplements with improved antioxidant efficacy on DS-induced cognitive impairment.

### Accumulation of Copper in the Brain of Ts1Cje Mice

As mentioned above, the association of certain Hsa21 genes with enhanced OS in DS has been mostly suggested based on observations obtained in overexpression experiments. While several Hsa21 genes may cooperatively exacerbate OS, other factors might also be involved in the enhanced OS in DS. Redox-active biometals, such as iron and copper, play a role in oxidative metabolism.<sup>(30)</sup> Dyshomeostasis of Fe(II/III) and Cu(I/II) may promote OS in DS. Indeed, the copper concentration in red blood cells was shown to be higher in people with DS than in age- and sex-matched controls without DS.<sup>(31)</sup>

Although iron promotes the release and metabolism of dopamine and other neurotransmitters,<sup>(32)</sup> excessive iron promotes ROS production through the Fenton reaction, which causes protein oxidation, lipid peroxidation, and DNA damage.<sup>(33)</sup> Abnormal iron concentrations have not yet been shown in the brains of DS patients, but increased levels of non-protein-bound iron and a slight reduction in the total serum iron content have been detected in both plasma and erythrocytes of people with DS.<sup>(34)</sup> Furthermore, an increase in the level of non-protein-bound iron was shown to be correlated with increased lipid peroxidation and cognitive decline.<sup>(35)</sup>

In addition to iron, copper is also biochemically redox active. Copper induces OS through two possible mechanisms: the Fenton reaction and downregulation of glutathione.<sup>(36)</sup> In the rat brain, copper-overload induces oxidative damage via decreased levels of glutathione and increased levels of MDA, a typical lipid peroxidation product.<sup>(37)</sup> Thus, copper plays a key role in the induction of OS of the brain. It has been shown that the copper content is increased in erythrocytes and the tongue muscles of individuals with DS,<sup>(38,39)</sup> suggesting that the overall copper level was increased; however, the number of samples in those studies was limited, and no studies have analyzed brain tissue, even in mouse models. Therefore, we measured the amounts of biogenic elements in the brain of the Ts1Cje, a mouse model of DS. Inductively coupled plasma mass spectrometry (ICP-MS) revealed elevated concentrations of copper in the brain of Ts1Cje mice.<sup>(40)</sup> Furthermore, we demonstrated that enhanced OS (as assessed by lipid peroxidation) and reduced anxiety-like behaviors were improved in Ts1Cje mice fed a low-copper diet.<sup>(40)</sup> Thus, the accumulation of copper in the brain of Ts1Cje mice seems to have caused enhancement of OS and reduced anxiety behaviors.

Regarding the relationship between decreased anxiety and enhanced OS, Hovatta *et al.*<sup>(41)</sup> identified several genes with a differential expression among six inbred mice. Among these genes, they found that increased activities of two antioxidant genes, glyoxalase-1 (Glo1) and glutathione reductase-1, were associated with increased anxiety in mice.<sup>(41)</sup> In contrast, it has

also been suggested that the expression of Glo1 may reflect the anxiety level, with a high Glo1 expression indicating a low anxiety level.<sup>(42)</sup> Although controversial observations have been reported, anxiety seems to have a profound connection with OS.

### Possibility of Cognitive Impairment by a High Concentration of Copper in the Brain

Wilson's disease, which is an inherited disorder induced by the accumulation of copper in the liver, brain, and other organs, is characterized by memory impairment and depression.<sup>(43)</sup> Furthermore, perturbations in brain concentrations of copper and zinc are suggested to underlie the pathoetiology of AD.<sup>(44,45)</sup> It has also been noted that individuals fed a diet high in saturated and trans fats who have high copper levels develop cognitive decline at a faster rate than others.<sup>(46)</sup> These observations suggest that a disturbed copper concentration in the brain can impair the cognitive function.

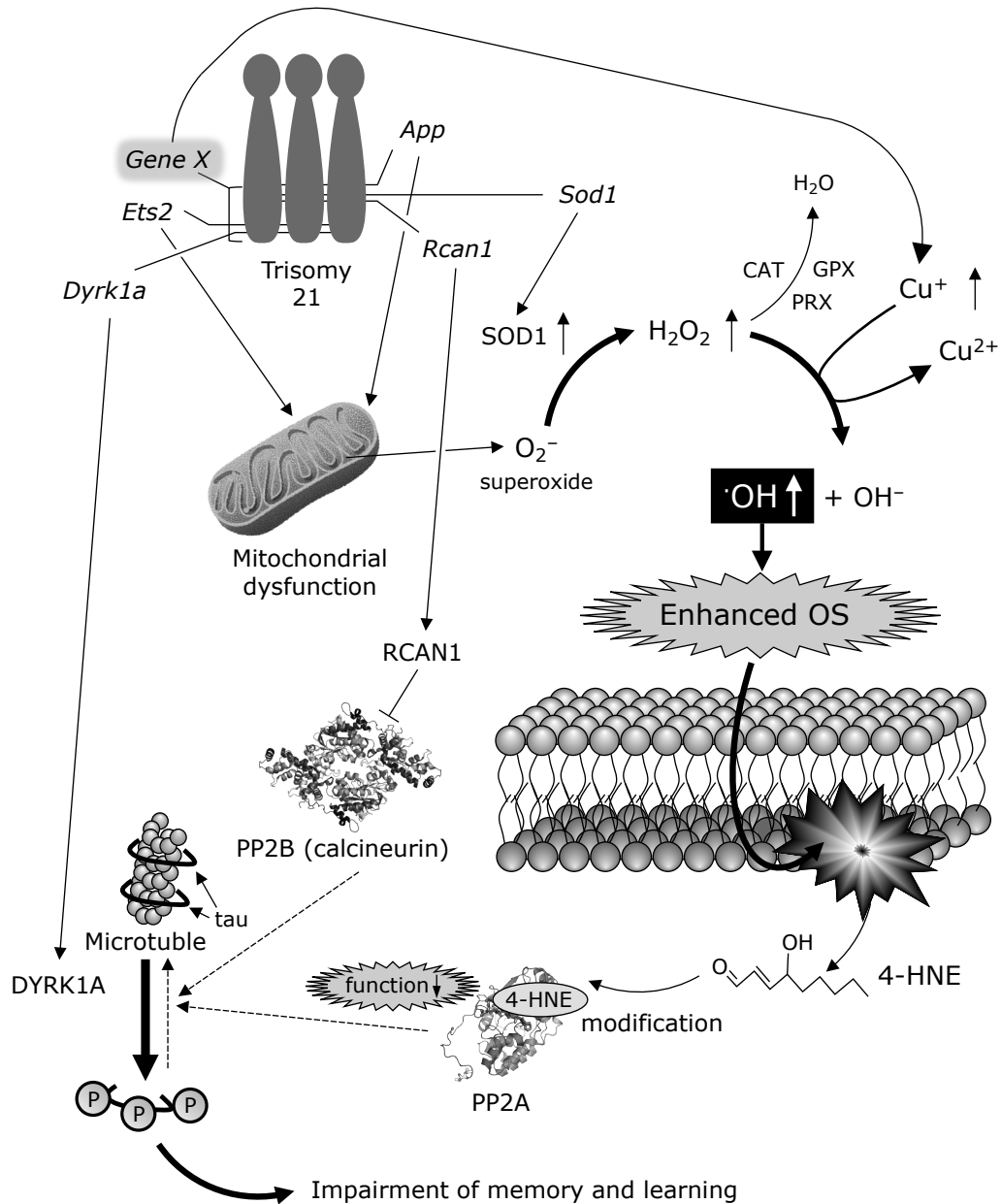
The accumulation of hyperphosphorylated tau has been shown in mouse models of DS,<sup>(15,47)</sup> such as Ts1Cje and Tc1 mice, which carry an almost complete, freely segregating copy of Hsa21.<sup>(48)</sup> Dyrk1a has been suggested to be a candidate for hyperphosphorylated tau deposition in multiple studies.<sup>(49-51)</sup> An *in vitro* study demonstrated that tau is phosphorylated at Thr212—which is suggested to be associated with the pathogenesis of AD—by DYRK1A.<sup>(49,52,53)</sup> A recent study found that enhanced OS negatively affected the function of protein phosphatase 2A (PP2A) through the modification of 4-hydroxy-2-nonenal (4-HNE), resulting in a reduction in the dephosphorylation of hyperphosphorylated tau in Ts65Dn mice.<sup>(54)</sup> Calcineurin, known as protein phosphatase 2B (PP2B), also dephosphorylates hyperphosphorylated tau protein.<sup>(55)</sup> Inhibition of calcineurin by RCAN1 overexpression in DS may contribute to the accumulation of hyperphosphorylated tau.

Copper promotes hyperphosphorylation and the aggregation of tau protein.<sup>(56)</sup> In addition, high copper accumulation has been detected in neurofibrillary tangles (NFTs).<sup>(57)</sup> In hTau mice expressing wild-type human tau and lacking endogenous mouse tau, exposure to high amounts of copper increased tau hyperphosphorylation without the accumulation of A $\beta$  and induced the impairment of spatial learning and memory.<sup>(58)</sup> Thus, the accumulation of copper in the brain with DS may lead to cognitive impairment via tau hyperphosphorylation.

Consistently, hyperphosphorylated tau accumulates in the hippocampus of Ts1Cje mice overexpressing DYRK1A.<sup>(15)</sup> Given our recent results showing that the accumulation of copper causes enhanced OS and the accumulation of hyperphosphorylated tau in Ts1Cje mice,<sup>(40)</sup> several genes coded in the trisomic region of Ts1Cje appear to enhance OS through the accumulation of copper, and the enhanced OS then induces the accumulation of 4-HNE-adducted PP2A. 4-HNE-modification would decrease the activity of PP2A, resulting in decrease of dephosphorylation of the hyperphosphorylated tau (Fig. 2). The trisomic region of the Ts1Cje mouse model codes no genes, which is suggested to associate with copper metabolism, so we assume that gene *X* indirectly upregulates the concentration of copper in the brain of DS individuals (Fig. 2).

### Conclusions

Enhanced OS may be involved in the pathophysiology of various DS anomalies, including cognitive dysfunction. We showed that the accumulation of copper causes enhanced OS in the brain of Ts1Cje mice, a DS mouse model.<sup>(40)</sup> Although clinical trials concerning treatment with the antioxidant vitamin E for cognitive impairment in individuals with DS have been performed in elderly people with DS, no beneficial effects on the progression of cognitive deterioration have been detected.<sup>(28)</sup> As



**Fig. 2.** Possible mechanisms underlying exacerbation of OS and the accumulation of phosphorylated tau in the brain of DS subjects. In DS brain, the gene-dosage-dependent increased expression of APP and ETS2 is suggested to lead to mitochondrial dysfunction and generate ROS, such as superoxide. The expression of SOD1 is also increased in a gene-dosage-dependent manner. An increased level of SOD1 leads to the production of H<sub>2</sub>O<sub>2</sub>. Gene X in Hsa21, which causes the accumulation of copper in the brain with DS, induces the production of hydroxy radicals, and the resulting enhanced OS produces 4-HNE through lipid peroxidation. Intercellular protein phosphatase 2A (PP2A), which dephosphorylates phosphorylated tau, is modified by 4-HNE and reduces its function. The DYRK1A level is upregulated in DS due to increased copy numbers. Since DYRK1A phosphorylates tau protein, the accumulation of phosphorylated tau in the DS brain is promoted. The increased expression of RCAN1 inhibits the activity of calcineurin (PP2B), which also dephosphorylates Tau protein. The accumulation of hyperphosphorylated tau in the DS brain may cause the impairment of memory and learning. A dashed arrows means a suppressed influences over the reaction.

shown in Fig. 2, OS enhanced by the accumulation of copper and disturbance of the tau kinase DYRK1A and the tau phosphatases PP2A and PP2B lead to the accumulation of hyperphosphorylated tau in the brain. This may be involved in the early onset of AD-like dementia in individuals with DS. Taken together, these findings suggest that copper is a promising target for pharmacotherapy of the cognitive impairment characteristic of DS, and indeed, a copper chelator has already been used to treat patients with Wilson's disease.

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## Abbreviations

AD	Alzheimer's disease
APP	amyloid precursor protein
CAT	catalase
DS	Down syndrome
DYRK1A	dual-specificity tyrosine phosphorylation regulated kinase 1A
Glo1	glyoxalase-1
GPX	glutathione peroxidase
Hsa21	human chromosome 21
4-HNE	4-hydroxy-2-nonenal
ICP-MS	inductively coupled plasma mass spectrometry
MDA	malondialdehyde

Mmu16	mouse chromosome 16
NFTs	neurofibrillary tangles
OS	oxidative stress
PP2A	protein phosphatase 2A
PP2B	protein phosphatase 2B
RCAN1	regulator of calcineurin-1
RNS	reactive nitrogen species
ROS	reactive oxygen species
SOD1	superoxide dismutase 1

## Conflict of Interest

No potential conflicts of interest were disclosed.

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