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Copper/Zinc Superoxide Dismutase Expression in the Human Central Nervous System

Correlation with Selective Neuronal Vulnerability

Catherine Bergeron,*† Connie Petrunka,* and Luitgard Weyer*

From the Centre for Research in Neurodegenerative Diseases and Department of Pathology, University of Toronto; and Department of Pathology,† The Toronto Hospital, Toronto, Ontario, Canada*

Oxidative stress has been implicated in the pathogenesis of several neurological disorders. We examined the regional distribution of copper/zinc superoxide dismutase (SOD-1), one of the key antioxidant enzymes, in the human central nervous system using* in situ *hybridization. Our results show that the enzyme is present at high levels of constitutive expression in α-motor neurons, oculomotor neurons, nucleus basalis, substantia nigra, neocortex, and the hippocampal sector resistant to hypoxia (H2). Relatively lower levels were found in Sommer's sector (H1) and Purkinje cells. We conclude that a lower constitutive level of SOD-1 expression may play a role in the selective vulnerability of certain neuronal populations to hypoxia but does not correlate with the patterns of neurodegeneration observed in amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. (Am J Pathol 1996, 148:273–279)

Oxidative stress has been implicated in the pathogenesis of several neurological disorders such as cerebral ischemia[1] and idiopathic Parkinson's disease (PD).[2] Copper/zinc superoxide dismutase (SOD-1) mutations have been discovered in several amyotrophic lateral sclerosis (ALS) families,[3–13] whereas SOD-1 induction,[14] increased protein carbamylation,[15] and upregulated glutathione receptors[16] suggest that oxidative stress may also be implicated in the sporadic form of the disease. Furthermore, Mitchell et al[17] demonstrated a decline in whole blood glutathione peroxidase activity in sporadic ALS, which they speculate may reflect increased mobilization and transport of the enzyme as a result of enhanced free radical activity. Finally, β-amyloid[18–20] and tau glycation[21,22] have been postulated as possible sources of reactive oxygen species in Alzheimer's disease (AD); increased protein carbamylation[23] and lipid peroxidation[24] as well as heme oxygenase-1 induction[25] are also observed in AD, further supporting the role of oxidative stress in this disease. Each of these disorders is characterized by the selective vulnerability of specific neuronal subsets that display different metabolic profiles. On this basis, one may speculate that certain neuronal populations may be more vulnerable to oxidative stress as a result of a greater oxidative burden or, alternatively, lower antioxidant protection. To further understand the possible role of oxidative stress and the cellular basis of selective neuronal death, we measured the expression of one of the key antioxidant enzymes, the copper/zinc form of superoxide dismutase (SOD-1), in several regions of the human brain and spinal cord. We further correlated our measurements with the metabolic characteristics of each neuronal subset analyzed, as well as their individual disease vulnerability.

Materials and Methods

Patient Population

Seven subjects with no evidence of neurological disease or neuropathology on histological examination were obtained for study. Details of the cases are provided in Table 1.

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Address reprint requests to Catherine Bergeron M. D., Centre for Research in Neurodegenerative Diseases, University of Toronto, 6 Queen's Park Crescent West, Toronto, Ontario, Canada, M5S 1A8.

Table 1. *Characteristics of the Cases Used for the Study*

Case no.	Age	Postmortem interval (hours)	Formalin fixation time (days)	Cause of death
C01	45	7	18	Respiratory failure
C02	25	7	12	Strangulation
C08	67	6	20	Myocardial Infarction
C11	72	9	17	Myocardial Infarction
C13	75	7	16	Sepsis
C14	73	10	22	Myocardial Infarction
C16	72	15	22	Myocardial Infarction

Selection of Brain Regions

The following blocks were selected for analysis: spinal cord at the L4–5 level, cerebellum, midbrain at the level of the third cranial nerve nucleus except for one case at the level of the fourth, hippocampus and middle temporal gyrus at the level of the lateral geniculate body, and nucleus basalis at the level of the posterior border of the anterior commissure. In each case, the following neuronal populations were studied: α-motor neurons, oculomotor nucleus motor neurons (trochlear nucleus in one case), Purkinje cells, pigmented dopaminergic neurons of the middle third of the substantia nigra pars compacta, pyramidal neurons of the hippocampus in both Sommer's sector (H1) and the hypoxia resistant sector (H2), pyramidal neurons of the fifth cortical layer, and nucleus basalis of Meynert.

In Situ *Hybridization*

A human SOD-1 cDNA coding for the entire SOD-1 protein was generously given by Dr. Y. Groner.[26] This probe is well characterized and has been used for *in situ* hybridization (ISH) studies of AD.[27,28] The probe was provided in plasmid form and inserted in the expression vector BRLpT3/T7 (Bethesda Research Laboratories, Bethesda, MD). The plasmid was linearized with *Hin*dIII (sense mRNA) and *Bam*HI (antisense mRNA) and transcribed *in vitro* using T3 and T7 polymerases with tritiated nucleotides. Transcripts were isolated by phenol extraction and repeated ethanol precipitations, and reduced to a length of 50 to 150 bases by limited alkaline hydrolysis.[29] Paraffin sections 5 μm thick were cut and mounted onto Denhardt's-treated slides under RNAse-free conditions. The slides were deparaffinized, rehydrated in graded alcohols, and pretreated with 10 μg/ml proteinase K for 10 minutes at 37°C. ISH was performed as previously described,[28] with an exposure time of 56 days. Controls for probe specificity included hybridization with the sense strand and hybridization after RNAse treatment. All slides from a given case were hybridized simultaneously to maintain constant experimental conditions.

Semiquantitative Analysis

A total of 20 consecutive neurons were analyzed in each region at a magnification of ×630. A LECO image analysis system (L2001, LECO Instruments, Montreal, Quebec, Canada) was used to count grains on the basis of color detection. Briefly, after a neuron is identified and the color threshold optimized for grain detection, it is manually outlined. The computer then automatically counts the grains within the outlined area. Further operations include a chord sizing step to eliminate features smaller than two pixels, and five erosion cycles to resolve individual grains. Because of color overlap between melanin and silver grains we have modified our image analysis routine for pigmented neurons. Briefly, after a pigmented neuron is selected, the melanin content is first outlined, as well as the cross-sectional area. The color threshold is then optimized for grain detection and, through a series of Boolean operations, the grain count is performed on the melanin-free cytoplasm. Background determinations are performed on 10 400 μm^2 areas of neuropil. Grain counts are expressed as grain density per μm^2 after background correction.

Several factors may influence the postmortem determination of mRNA levels in human brain using ISH, including age, postmortem interval, tissue fixation, and agonal status.[30] In our study, all regions from a given case were subjected to similar conditions and were hybridized simultaneously to maintain uniform and constant experimental conditions. We were therefore able to conduct a region-to-region comparison using the pooled regional values for each case using Student's unpaired two-tailed *t*-tests.

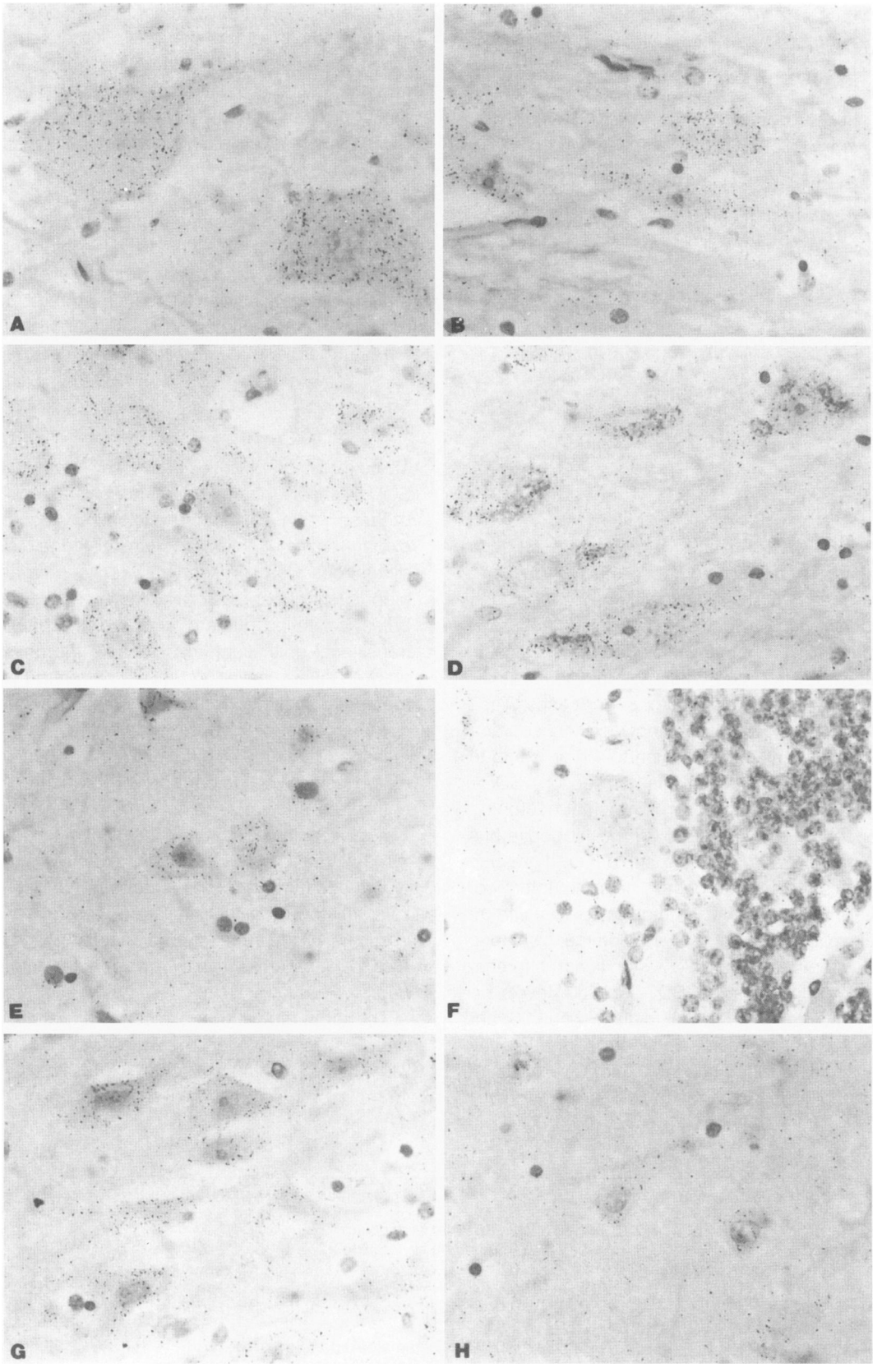

Figure 1. *SOD-1 mRNA* in situ *hybridization grains in selected brain regions.* (**A**) *α-Motor neurons.* (**B**) *Oculomotor nucleus.* (**C**) *Nucleus basalis.* (**D**) *Substantia nigra, pars compacta.* (**E**) *Temporal neocortex, fifth layer.* (**F**) *Purkinje cells.* (**G**) *Hippocampus, H2.* (**H**) *Hippocampus, H1. Case C02, Hematoxylin/eosin counterstain, original magnification × 100.*

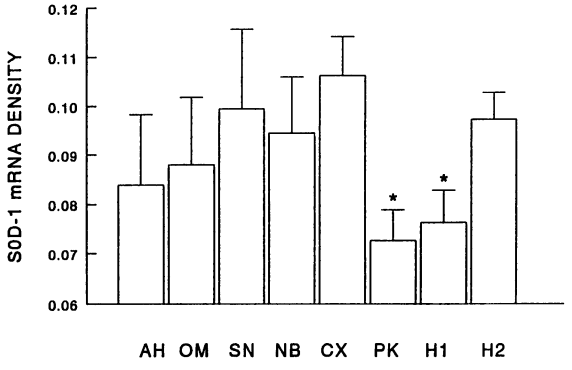

Figure 2. *SOD-1 mRNA levels are high in all neuronal populations tested, but consistently lower in Sommer's sector and Purkinje cells (two tailed unpaired Student's t-test, *P < 0.03). Pooled mean regional SOD-1 mRNA levels expressed in grains/μm². AH = α-motor neurons; OM = oculomotor nucleus; SN = substantia nigra, pars compacta; NB = nucleus basalis; CX = pyramidal neurons, fifth layer, neocortex, temporal lobe; PK = Purkinje cells; H1 = hippocampus, H1 (Sommer's sector); H2 = hippocampus, H2 (hypoxia-resistant sector).*

Results

SOD-1 mRNA hybridization grains were found in high numbers in the cytoplasm and proximal processes of all neurons examined; an axonal or dendritic localization could not be recognized (Figure 1). Regional SOD-1 mRNA levels varied between cases as anticipated from the difference in age, postmortem interval, and agonal status in our cases (Table 1). As previously discussed, however, these variations would not affect our region-to-region comparisons (see Semiquantitative Analysis section). SOD-1 mRNA levels were highest in pyramidal neurons of the neocortex and H2 sector of the hippocampus, dopaminergic neurons of the substantia nigra pars compacta, and nucleus basalis neurons. Intermediate levels were observed in motor neurons with the lowest levels in Purkinje cells and Sommer's sector of the hippocampus (Figure 2). A significant difference was seen in the neuronal SOD-1 mRNA levels between the cortex and Purkinje cells ($P = 0.006$) and H1 ($P = 0.013$), as well as between H2 and Purkinje cells ($P = 0.013$) and H1 ($P = 0.023$). There was no correlation between the mean SOD-1 mRNA levels and the profile of the neuronal populations studied, including those factors that could increase the neuronal oxidative burden such as the type of transmitter, the metabolic rate, or *N*-methyl-D-aspartate (NMDA) receptor density (Table 2). Likewise, mean SOD-1 mRNA levels did not correlate with the pattern of selective neuronal vulnerability for AD, ALS, or PD, but the lowest SOD-1 levels were observed in two of the brain regions most susceptible to hypoxia, Purkinje cells and the H1 sector of the hippocampus (Table 3). Few hybridization grains were observed after hybridization with the sense strand, and the hybridization signal was completely abolished after RNAse treatment, as previously illustrated.[28]

Discussion

The expression of antioxidative enzymes, including SOD-1, shows marked variation between and within organs.[31,32] In an immunohistological study of antioxidant enzymes in Syrian hamster kidney, marked differences were present between cell types, with high levels of expression in proximal and distal tubules, collecting ducts of the tip of the papilla and transitional epithelium; in this case, the antioxidant enzyme levels, including SOD-1, correlated with the metabolic profile, function, and disease vulnerability of the different cell types.[32] For example, the proximal and distal tubules display a high level of aerobic metabolism, while the papillary ducts and transitional epithelium are exposed to high concentrations of xenobiotics. In contrast, low levels of antioxidant enzymes in the glomerulus may explain the sensitivity of this structure to oxidative stress in the course of inflammation, and the similarly low levels of these enzymes in the renal papilla may predispose this area to injury by reactive oxygen species and lead to papillary necrosis.[32]

The brain also shows a differential pattern of expression of antioxidant enzymes along cell types with a predominant neuronal expression of SOD-1.[33,34] In contrast, glutathione peroxidase and gluta-

Table 2. *Profile of the Selected Neurons*

Region	SOD-1*	Transmitter	NMDA receptors	Metabolic rate
Spinal cord	0.084 ± 0.038	Acetylcholine	Low	High
Oculomotor	0.088 ± 0.037	Acetylcholine	Low	High
Substantia nigra (pars compacta)	0.099 ± 0.039	Dopamine	Low	Low
Nucleus basalis	0.094 ± 0.030	Acetylcholine	N/A	N/A
Cortex, fifth layer	0.106 ± 0.021	Glutamate/Aspartate	High	High
Purkinje cells	0.073 ± 0.017	GABA	Low	High
Hippocampus, H1	0.076 ± 0.017	Glutamate/Aspartate	High	Low
Hippocampus, H2	0.097 ± 0.014	Glutamate/Aspartate	Moderate	High

*Pooled mean regional SOD-1 mRNA levels expressed in grains/μm².

Table 3. *Disease Vulnerability of Selected Neuronal Areas*

Region	SOD-1*	ALS	AD	PD	Hypoxia
Spinal cord	0.084 ± 0.038	High	0	0	+/−
Oculomotor	0.088 ± 0.037	Low	0	+/−	Low
Substantia nigra (pars compacta)	0.099 ± 0.039	+/−	+/−	High	0
Nucleus basalis	0.094 ± 0.030	0	High	High	0
Cortex, fifth layer	0.106 ± 0.021	0	High	0	Moderate
Purkinje cells	0.073 ± 0.017	0	0	0	High
Hippocampus, H1	0.076 ± 0.014	0	High	0	High
Hippocampus, H2	0.097 ± 0.014	0	Low	0	Moderate

*Pooled mean regional SOD-1 mRNA levels expressed in grains/μm^2.

thione are exclusively astrocytic,[35–38] while SOD-2 is present in both neurons and astrocytes.[39] Our results show, however, that neurons as a whole exhibit high levels of SOD-1 with only minor regional variations, consistent with the recent immunohistochemical study of Pardo et al.[40]

Unlike the situation observed in the kidney, metabolic differences between neurons correlate poorly with SOD-1 expression (Table 2). One may predict a higher expression in dopaminergic neurons where the auto-oxidation of dopamine and its metabolism by monoamine oxidase produces free radicals, therefore exposing the cells to a larger oxidative burden.[41] Neuronal population with a high density of NMDA receptors[42] may also be expected to display higher constitutive SOD-1 levels in view of their predisposition to excitotoxic damage and subsequent free radical production. The relatively uniform expression of SOD-1 may result from the fact that all neurons share a similarly high level of aerobic metabolism[43] and that, notwithstanding regional differences,[44] they perform a single function, that of signaling.

Our results show clearly that the constitutive level of SOD-1 expression does not underlie the pattern of neuronal selectivity to neurodegeneration (Table 3). In ALS, eg, α-motor neurons are most vulnerable to degeneration, while motor neurons of the oculomotor nucleus are relatively resistant and other cell types are virtually unaffected.[45] This pattern of vulnerability is certainly not explained by the constitutive expression of SOD-1 and must therefore result from other, as yet undefined factors. A similar lack of correlation is also observed for AD[46] and idiopathic PD[47] (Table 3). In fact, neuronal populations exposed to an increased oxidative burden appear able to compensate for this insult by upregulating the expression of antioxidant enzymes such as SOD-1 in sporadic ALS,[14] heme oxygenase-1 in AD,[25] and SOD-1 and SOD-2 in PD.[48,49] In contrast, consistently lower SOD-1 levels are observed in Sommer's sector (H1) and Purkinje cells of the cerebellum, a finding that may contribute to their marked sensitivity to acute ischemic-hypoxic injury.[50]

The selective vulnerability of neurons to hypoxia and neurodegeneration is likely to result from the interaction of several factors including the ability of the neurons to cope with oxidant stresses. The latter in turn depends on the interaction of several antioxidant enzymes and molecules including SOD-1. Further studies are needed to determine the status of other antioxidant molecules in the human brain in health and disease, as well as other metabolic factors that may predispose selected neuronal populations to cellular dysfunction and death.

References

1. Floyd RA: Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J 1990, 4:2587–2597
2. Jenner P: Oxidative damage in neurodegenerative disease. Lancet 1994, 344:796–798
3. Deng H-X, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung W-Y, Getzoff ED, Hu P, Herzfeldt B, Roos RP, Warner C, Deng G, Soriano E, Smyth C, Parge HE, Ahmed A, Roses AD, Hallewell RA, Pericak-Vance MA, Siddique T: Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science 1993, 261:1047–1051
4. Ogasawara M, Matsubara Y, Narisawa K, Aoki M, Nakamura S, Abe K: Mild ALS in Japan associated with novel SOD mutation. Nature Genet 1993, 5:323–324
5. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, et al: Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993, 362:59–62
6. Aoki M, Ogasawara M, Matsubara Y, Narisawa K, Nakamura S, Itoyama Y, Abe K: Familial amyotrophic lateral sclerosis (ALS) in Japan associated with a H46R mutation in Cu/Zn superoxide dismutase gene: a possible new subtype of familial ALS. J Neurol Sci 1994, 126:77–83

7. Elshafey A, Lanyon GW, Connor MJ: Identification of a new missense point mutation in exon 4 of the Cu/Zn superoxide dismutase (SOD-1) gene in a family with amyotrophic lateral sclerosis. Hum Mol Genet 1994, 3:363–364
8. Esteban J, Rosen DR, Bowling AC, Sapp P, McKenna-Yasek D, O'Regan JP, Flint Beal M, Horvitz HR, Brown RHJ: Identification of two novel mutations and a new polymorphism in the gene for Cu/Zn superoxide dismutase in patients with amyotrophic lateral sclerosis. Hum Mol Genet 1994, 3:997–998
9. Hirano M, Fujii J, Nagai Y, Sonobe M, Okamato K, Araki H, Taniguchi N, Ueno S: A new variant Cu/Zn superoxide dismutase (Val^7 Glu) deduced from lymphocyte mRNA sequences from Japanese patients with familial amyotrophic lateral sclerosis. Biochem Biophys Res Commun 1994, 204:572–577
10. Kawamata J, Hasegawa H, Shimohama S, Kimura J, Tanaka S, Ueda K: Leu106 Val (CTC GTC) mutation of superoxide dismutase-1 gene in patient with familial amyotrophic lateral sclerosis in Japan. Lancet 1994, 343:1501
11. Nakano R, Sato S, Inuzuka T, Sakimura K, Mishina M, Takahashi H, Ikuta F, Honma Y, Fujii J, Taniguchi N, Tsuji S: A novel mutation in Cu/Zn superoxide dismutase gene in Japanese familial amyotrophic lateral sclerosis. Biochem Biophys Res Commun 1994, 200: 695–703
12. Pramatarova A, Goto J, Nanma E, Nakashima K, Takahashi K, Takaji A, Kanazawa I, Figlewicz DA, Rouleau GA: A two basepair deletion in the SOD 1 gene causes familial amyotrophic lateral sclerosis. Hum Mol Genet 1994, 3:2016–2062
13. Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, Mckenna-Yasek D, O'Regan J, Rahmani Z, Ferrante RJ, Brownstein MJ, Kowall NW, Flint Beal M, Horvitz HR, Brown RHJ: A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis. Hum Mol Genet 1994, 3:981–987
14. Bergeron C, Muntasser S, Somerville MJ, Weyer L, Percy ME: Copper/Zinc superoxide dismutase mRNA levels are increased in sporadic amyotrophic lateral sclerosis motorneurons. Brain Res 1994, 659:272–276
15. Bowling AC, Schulz JB, Brown RH, Beal MF: Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J Neurochem 1993, 61: 2322–2325
16. Lanius R, Krieger C, Wagey R, Shaw C: Increased [^{35}S]glutathione binding sites in spinal cords from patients with sporadic amyotrophic lateral. Neurosci Lett 1993, 163:89–92
17. Mitchell J, Gatt J, Phillips T, Houghton E, Roston G, Wignall C: Cu/Zn superoxide dismutase free radical, and motorneurone disease. Lancet 1993, 342:1051–1052
18. Behl C, Davis J, Lesley R, Schubert D: Hydrogen peroxide mediates amyloid β protein toxicity. Cell 1994, 77:817–827
19. Butterfield DA, Hensley K, Harris M, Mattson M, Carvey J: β-amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. Biochem Biophys Res Commun 1994, 200:710–715
20. Hensley K, Carney J, Mattson M, Aksenova M, Harris M, Wu J, Floyd R, Butterfield D: A model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc Natl Acad Sci USA 1994, 91:3270–3274
21. Smith MA, Taneda S, Richey PL, Miyata S, Yan S-D, Stern D, Sayre LM, Monnier VM, Perry G: Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proc Natl Acad Sci USA 1994, 91:5710–5714
22. Yan S-D, Chen X, Schmidt A-M, Brett J, Godman G, Zou Y-S, Scott C, Caputo C, Frappier T, Smith M, Perry G, Yen S-H, Stern D: Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci USA 1994, 91:7787–7791
23. Smith C, Carney J, Starke-Reed P, Oliver C, Stadtman E, Floyd R, Markesbery W: Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 1991, 88: 10540–10543
24. Subbarao KV, Richardson JS, Ang LC: Autopsy samples of Alzheimer's cortex show increased peroxidation *in vitro*. J Neurochem 1990, 55:342–345
25. Smith MA, Kutty RK, Richey PL, Yan S-D, Stern D, Chader GJ, Wiggert B, Petersen R, Perry G: Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. Am J Pathol 1994, 145: 42–47
26. Groner Y, Lieman-Hurwitz J, Dafni N, Sherman L, Levanon D, Bernstein Y, Danciger E, Elroy-Stein O: Molecular structure and expression of the gene locus on chromosome 21 encoding the Cu/Zn superoxide dismutase and its relevance to Down's syndrome. Molecular Structure of the Number 21 Chromosome and Down Syndrome. Edited by GF Smith. Ann NY Acad Sci 1985, 450:133–156.
27. Somerville MJ, Bergeron C, Yoong LK, McLachlan DR, Percy ME: Neuronal expression of the light chain neurofilament and superoxide dismutase-1 genes in temporal lobe of Alzheimer brain. Alzheimer's disease: basic mechanisms, diagnosis and therapeutic strategies. Edited by K Iqbal, D McLachlan, B Windblad, H Wisniewski. New York, John Wiley and Sons Ltd, 1991: 237–241
28. Somerville MJ, Percy ME, Bergeron C, Yoong LKK, Grima EA, McLachlan DRC: Localization and quantitation of 68 kDA neurofilament and superoxide dismutase-1 mRNA in Alzheimer brain. Mol Brain Res 1991, 9:1–8
29. Pardue ML: *In situ* hybridization. Nucleic Acid

Hybridization: A Practical Approach. Edited by BD Hames, SJ Higgins. Oxford, IRL Press, 1985: 179–202
30. Barton A, Pearson R, Najlerahim A, Harrison P: Pre- and postmortem influences on brain RNA. J Neurochem 1993, 61:1–11
31. Oberley TD, Oberley LW, Slattery AF, Lauchner LJ, Elwell JH: Immunohistochemical localization of antioxidant enzymes in adult syrian hamster tissues and during kidney development. Am J Pathol 1990, 137:199–214
32. Muse KE, Oberley TD, Sempf JM, Oberley LW: Immunolocalization of antioxidant enzymes in adult hamster kidney. Histochem J 1994, 26:734–753
33. Ceballos I, Javoy-Agid F, Delacourte A, Defossez A, Lafon M, Hirsch E, Nicole A, Sinet P, Agid Y: Neuronal localization of copper-zinc superoxide dismutase protein and mRNA within the human hippocampus from control and Alzheimer's disease brains. Free Rad Res Commun 1991, 12:571–580
34. Zhang P, Damier P, Hirsh E, Agid Y, Ceballos-Picot I, Sinet P, Nicole A, Laurent M, Javoy-Agid F: Preferential expression of superoxide dismutase messenger RNA in melanized neurons of human mesencephalon. Neuroscience 1993, 55:167–175
35. Slivka A, Mytilineou C, Cohen G: Histochemical evaluation of glutathione in brain. Brain Res 1987, 409:275–284
36. Raps SP, Lai JC, Hertz L, Cooper AJ: Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. Brain Res 1989, 493:398–401
37. Damier P, Hirsch E, Zhang P, Agid Y, Javoy-Agid F: Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 1993, 52:1–6
38. Makar TK, Nedergaard M, Preuss A, Gelbard A, Perumal A, Cooper AJ: Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes, and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain. J Neurochem 1994, 62:45–53
39. Zhang P, Anglade P, Hirsch E, Javoy-Agid F, Agid Y: Distribution of manganese-dependent superoxide dismutase in the human brain. Neuroscience 1994, 61: 317–330
40. Pardo CA, Xu Z, Borchelt DR, Price DL, Sisodia S, Cleveland DW: Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. Proc Natl Acad Sci USA 1995, 92:954–958
41. Olanow C: An introduction to the free radical hypothesis in Parkinson's disease. Ann Neurol 1992, 32:S2–9
42. Monaghan DT, Cotman CW: Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. J Neurosci 1985, 5:2909–2919
43. Clarke DD, Sokoloff L: Circulation and energy metabolism of the brain. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, ed 5. Edited by GJ Siegel, BW Agranoff, RW Albers, PB Molinoff. New York, Raven Press, 1994
44. Friede RL, Fleming LM: A mapping of oxidative enzymes in the human brain. J Neurochem 1962, 9:179–198
45. Chou SM: Pathology–Light Microscopy of Amyotrophic Lateral Sclerosis: Handbook of Amyotrophic Lateral Sclerosis. Edited by RA Smith. New York, Marcel Dekker, Inc., 1992, pp 133–181
46. Tomlinson B: Ageing and the dementias. Greenfield's Neuropathology, ed 5. Edited by JH Adams, LW Duchen. New York, Oxford University Press, 1992, pp 1284–1410
47. Oppenheimer D, Esiri MM: Diseases of the basal ganglia, cerebellum and motor neurons. Greenfield's Neuropathology, ed 5. Edited by JH Adams, LW Duchen. New York, Oxford University Press, 1992, pp 988-1045
48. Marttila R, Lorentz H, Rinne U: Oxygen toxicity protecting enzymes in Parkinson's disease. Increase of superoxide dismutase-like activity in the substantia nigra and basal nucleus. J Neurol Sci 1988, 86:321–331
49. Saggu H, Cooksey J, Dexter D, Wells F, Lees A, Jenner P, Marsden C: A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. J Neurochem 1989, 53:692–697
50. Graham D: Hypoxia and vascular disorders. Greenfield's Neuropathology, ed 5. Edited by JH Adams, LW Duchen. New York, Oxford University Press, 1992 pp 153–268