Is ALS caused by an altered oxidative activity of mutant superoxide dismutase?

TO THE EDITOR—Evidence indicates that Cu/Zn superoxide dismutase 1 (SOD1) mutations linked to amyotrophic lateral sclerosis (ALS) lead to inappropriate release of copper (Cu) redox activity and oxidative damage[1–3], which can be ameliorated by Cu chelators[4,5] and exacerbated by genetic ablation of copper-sequestering metallothioneins[6]. The recent study by Subramanian and colleagues[7] in *Nature Neuroscience* demonstrates that neurodegeneration in transgenic mice overexpressing mutant SOD1 is not rescued by genetic removal of the copper chaperone of SOD1 (CCS). This study convincingly showed that pathogenesis in the mouse model was not due to the activity of Cu at the active site of SOD1, as CCS is required for efficient loading of Cu into this structure[8]. The biological activity of the SOD1 enzyme is indeed relatively reduced in $CCS^{-/-}$/SOD1-mutant crossed mice[7].

However, these findings still do not allow us to disqualify Cu from the pathogenesis of this disorder. The view that "ALS is not caused by an altered oxidative activity of SOD1", proposed in the accompanying News and Views[9] in the same issue, is not conclusively supported by the findings, because a pro-oxidative interaction between Cu and SOD1 at sites other than the catalytic Cu binding site has not been excluded.

The pathological, Cu-dependent, pro-oxidant reactions attributed to SOD1 include peroxidation[1], peroxynitrite generation[2] and inappropriate hydrogen peroxide generation[3]. The pro-oxidant chemistry of mutant SOD1 has been sometimes incorrectly attributed to "the requirement of copper at the SOD1 catalytic site"[9]. This is a misconception because pro-oxidant (for example, peroxidative) activity is mediated by other, non-dismutase Cu binding sites on the protein.

There are at least two other documented Cu binding sites on SOD1 that have little or no SOD activity. Cu can incorrectly bind to the zinc binding site[10], and Cu can also bind to the surface residues of SOD1 (ref. 11). H46R mutant SOD1, linked to familial ALS, has no SOD activity but binds Cu at surface residue Cys111, a possible transition site for CCS-mediated copper loading[11]. The Cys111-bound Cu is redox-active and can catalyze deleterious pro-oxidant reactions. To show that CCS ablation abolishes Cu binding to these sites, a decrease in the Cu binding stoichiometry to mutant SOD1 would need to be demonstrated.

The data of Subramanian and colleagues do not establish such a decrease in the $CCS^{-/-}$ mice. The authors have taken the decrease in ^{64}Cu-radiolabeled SOD1 in $CCS^{-/-}$ mice, and accompanying decreased dismutase activity, as proof that Cu stoichiometry is diminished. However, without controls that correlate the dynamic incorporation of ^{64}Cu with the stoichiometric content of Cu bound to SOD1 in the tissue, it is inaccurate to conclude that chemical equilibrium had been reached, and that there was an "80% reduction" in the amount of total Cu bound to SOD1 in the $CCS^{-/-}$ mice[7]. The marked decrease in dismutase activity that was observed in the $CCS^{-/-}$/SOD1-mutant mice also does not prove that total Cu binding to SOD1 is decreased, as this activity is only a reflection of Cu binding at the active site; the dismutase activity assay does not reflect Cu bound to other sites on the protein.

What then accounts for the diminished ^{64}Cu incorporation into SOD1 in the $CCS^{-/-}$ mice? It is possible that CCS ablation forces Cu to dwell on the transition loading site of SOD1, which normally would be unoccupied[11]. This could cause a decrease in dismutase activity, whereas the stoichiometry of Cu binding would be unchanged. The occupied transition site would then act as a chemical log-jam, inhibiting further incorporation of ^{64}Cu. Supporting this possibility, the $CCS^{-/-}$ lesion alone caused a marked (and unexplained) elevation of total tissue Cu (Fig. 3d in ref. 7); this increased Cu could saturate binding sites on SOD1 and inhibit further uptake of ^{64}Cu. Other data in Fig. 3d show that the two strains of mutant SOD1 mice (with $CCS^{+/+}$) both had ~3.5 g/g (~30%) elevations in brain Cu levels compared to normal mice. This is in agreement with the significant increase in spinal cord Cu levels that we observed in G93A mutant SOD1 transgenic mice (C. Maynard *et al.*, unpublished data). This marked elevation of tissue Cu (~30 M) is probably Cu attached to the overexpressed SOD1. The $CCS^{-/-}$ lesion did not decrease total Cu levels in the mutant SOD1 tissue (Fig. 3d). Therefore, the ablation of CCS probably does not decrease Cu binding stoichiometry to mutant SOD1.

In summary, the Cu-mediated oxidative damage by mutant SOD1 is not predicted to be proportional to dismutase activity, but rather, proportional to peroxidative or other pro-oxidant activity, which was not assessed by Subramanian and colleagues. Their findings do not exclude Cu from the pathophysiology of ALS. However, we can now eliminate one of the Cu binding sites on SOD1 from the pathogenesis of ALS.

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REPLY—Although the molecular mechanisms whereby mutant SOD1 causes selective motor neuron death remain unclear, a prevalent hypothesis[1,2] is that the toxic property of mutant SOD1 might be related to mutation-induced conformational changes in SOD1 that result in aberrant oxidative activities, which are catalyzed by the copper atom that is bound in the active site of mutant SOD1. The realization that the copper chaperone for SOD1 (CCS) is necessary for efficient copper incorporation into SOD1 in yeast[12] and in mammals[8] encouraged us to directly test this copper theory *in vivo* by deleting the CCS in multiple lines of mutant SOD1 mice. The absence of CCS results in a signifi-