Title: Accumulation of dysfunctional SOD1 protein in Parkinson's disease is not associated with mutations in the *SOD1* gene

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In the first issue of Volume 134 of *Acta Neuropathologica*, we reported the substantial accumulation of abnormal deposits of superoxide dismutase 1 (SOD1) protein in the idiopathic Parkinson's disease brain, reflecting the pattern of neuronal loss in this disorder more closely than that of α-synuclein [10]. We presented evidence of catalytically dysfunctional, misfolded conformations of soluble and aggregated SOD1 protein in degenerating Parkinson's disease brain regions, similar to neurotoxic SOD1 proteinopathy in the spinal cord [8] and substantia nigra pars compacta (SNc) of familial amyotrophic lateral sclerosis (fALS) patients with mutations in the *SOD1* gene. Comparable changes in SOD1 structure and function suggest a common biochemical pathway contributing to neuron loss in both disorders. This provokes the question of whether mutant SOD1 is a feature of Parkinson's disease.

In the time since our report was published, we have conducted genotyping experiments on the 17 idiopathic Parkinson's disease cases in which we observed SOD1 dysfunction and aggregation to identify possible mutations in *SOD1,* using our previously reported methods for genetic profiling of SOD1 in fALS [7]. No sequence variations from wild type *SOD1* were identified in any of these cases of Parkinson's disease. This finding is consistent with the single study reported to date that failed to identify *SOD1* mutations in index familial Parkinson's disease patients representing 23 genealogies [1]. One participant in our Parkinson's disease cohort possessed a known intronic deletion found in healthy individuals (dbSNP rs398081559, c.573+88 del A), but did not represent an outlier within our

published datasets for SOD1 misfolding and deposition [10]. The absence of mutations in *SOD1* in our Parkinson's disease cohort indicates that aggregated SOD1 in these cases is wild type protein.

This negative result is important, as it demonstrates that wild-type, and mutant, SOD1 can express comparable dysfunctional activities and abnormal conformations in Parkinson's disease and fALS, respectively. These perturbations may represent a common basis for neuronal vulnerability in these disorders through a common molecular pathway that may involve either wild type or mutant SOD1.

The formation of a thermally stable SOD1 homodimer is essential for catalytic dismutation of superoxide to hydrogen peroxide and oxygen, mediated by two copper (II) ions. The binding of these copper (II) ions, along with the binding of two zinc (II) ions and the formation of an intramonomeric metal-stabilised disulfide bridge (Cys57-Cys146), affords the protein its exceptional thermodynamic stability. Consequently, reduced copper binding to SOD1 results in a profound destabilization of the protein and simultaneously prevents the permanent formation of the stabilizing intramonomeric disulfide bridge and the catalysis of superoxide [6]. Modification of any one of the protein's four cysteine residues [9], or tyrosine or histidine residues [11], can also result in an unstable protein prone to disordered oligomerisation. In cases of *SOD1* fALS , such perturbations may be attributable to the mutated protein but our current data indicate that, in idiopathic Parkinson's disease, such modifications occur following normal protein translation. Abnormal post-translational modifications of the wild type protein likely arise from a combination of substantially elevated intraneuronal oxidative stress and biometal dyshomeostasis characteristic of degenerating brain regions in Parkinson's disease [3, 10]. Importantly, despite a lack of concrete evidence of misfolded SOD1 in the more prevalent sporadic form of (s)ALS [2], these results support data demonstrating atypical post-translational modification of wild type SOD1 which may result in SOD1 dysfunction in sALS comparable to mutant SOD1 dysfunction in *SOD1* fALS [4].

In summary, we propose that a copper deficiency in SOD1, arising from either *SOD1* mutations that affect metal binding in fALS [5], or the generalised paucity of copper within catecholaminergic neurons we have previously reported in the Parkinson's disease brain [3], is directly associated with SOD1 misfolding and dysfunction [10]. The absence of mutations to *SOD1* in our Parkinson's disease cohort further justifies the conclusions we drew in our recent paper in *Acta Neuropathologica;* that a key endogenous mediator of oxidative stress in vulnerable catecholaminergic neurons is not only defective due to a lack of bioavailable copper, but is also susceptible to detrimental modifications that further impair metal retention, resulting in neurotoxic aggregation. This has important implications for the search for a tractable molecular 'trigger' of neurodegeneration in Parkinson's disease, but also for a potential role of wild type SOD1 dysfunction in sALS.

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AUTHOR CONTRIBUTIONS: B.G.T. and K.L.D. designed the study. B.G.T. and K.L.D. applied for all human tissues. B.G.T. and K.L.D. raised funds for the study. B.G.T. and K.L.D. gained human research ethics approval. S.J.G.L. and G.M.H. provided clinical information for all human tissue cases obtained. J.A.F, S.E.F and I.P.B performed the experiments and analyzed the data. B.G.T., D.J.H. and K.L.D. wrote drafts of the manuscript. All authors edited the manuscript.

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DATA AVAILABILITY: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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