Clinical/Scientific Notes

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A NOVEL α-SYNUCLEIN MISSENSE MUTATION IN PARKINSON DISEASE

α-Synuclein (SNCA) is central to the pathogenesis of Parkinson disease (PD), with 3 missense mutations reported to date. We report a novel mutation (p.H50Q) in a pathologically proven case.

Methods and results. ***Standard protocol approvals, registrations, and patient consents.*** Patients had given written informed consent for use of their brains in research. The study was approved by the local ethics committee.

Genetic analysis. We amplified and sequenced *SNCA* exons (ENSEMBL transcript ID 394986) in DNA extracted from substantia nigra (SN) of 5 Queen Square PD Brain Bank cases (primers given in table e-1 on the *Neurology*® Web site at www.neurology.org). We detected a point mutation in exon 3 in 1 case (c.150T>G) (figure e-1), causing a nonconservative missense change of the basic histidine to the polar but uncharged glutamine (p.H50Q). We confirmed it by bidirectional sequencing, repeat PCR, and sequencing using 2 different primer pairs and Phusion high-fidelity polymerase, sequencing of DNA from the cerebellum of the same patient, restriction digestion (figure e-2), and TOPO cloning of PCR products followed by PCR and restriction digestion of 30 bacterial colonies; 14 of these had the mutation, which was confirmed by sequencing (figure e-1).

Clinical information. The patient, a Caucasian English female, presented at age 71 years with right-sided tremor, responded to L-dopa, became forgetful at age 80, and died at age 83. There was no family history, but her parents' ages of death were not known. One of her 3 brothers had died of myocardial infarction, and the others, aged 74 and 64 years, and her daughter, were healthy. No relatives were available. Autopsy confirmed PD, with loss of pigmented cells in the SN, and Lewy bodies in the SN, midbrain pretectal area, and cerebral cortex. Plaques were noted in occipital and temporal cortex, with neurofibrillary tangles in the hippocampus, parahippocampal gyrus, and temporal cortex. Previous biochemical analysis demonstrated 44% reduction of mitochondrial complex I activity in the SN.[1]

Further evaluation and functional study of the mutation. The mutation was absent in the database of single nucleotide polymorphisms and 1,000 genomes, and we excluded it by restriction digestion in 450 control DNA samples from the Wellcome Trust 1958 British birth cohort (99.8% Caucasoid). Multiple alignment using CLUSTAL-W revealed conservation of H50 in SNCA orthologs in higher vertebrates, although in the American chameleon Q occurs naturally (figure 1A). Bioinformatic analysis using Mutation Taster (http://www.mutationtaster.org) and SNPS&GO (http://snps-and-sgo.biocomp.unibo.it/snps-and-go/) predicted pathogenicity. Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) predicted the mutation as possibly damaging (HumDiv score 0.797) or benign (HumVar score 0.452); notably, the A53T mutation is predicted as benign by Polyphen-2 (0 in both algorithms). Protein modeling using RaptorX (http://raptorx.uchicago.edu/) revealed that H50 is flanked within 1 turn of the α-helix by 2 known mutation sites, E46 and A53 (figure 1B).[1]

The H50 imidazole side chain is an avid copper (Cu^{2+}) binding group, and our previous work confirmed its participation in copper binding.[2] We investigated the effect of H50Q by electron paramagnetic resonance (EPR) using recombinantly expressed wild-type (wt) SNCA, H50Q, and the previously studied H50A, as described.[2] Comparison of EPR spectra from all, each with a single equivalent of Cu^{2+}, revealed greater splitting (larger A_{II}) and a downfield shift (larger g_{II}) of the parallel hyperfine region for H50Q relative to wt, similar to H50A, and consistent with replacement of an equatorial nitrogen with an oxygen (figure 1C, table e-2). Moreover, the H50Q spectrum did not overlap with that of wt. These data demonstrate that, in contrast to wt SNCA, H50Q takes up Cu^{2+} exclusively at its N terminus.

Discussion. We have detected a novel *SNCA* missense change that affects copper coordination in 1 apparently sporadic PD case. H50Q SNCA does bind Cu^{2+}, but there is no participation in metal coordination from other portions of the protein. This contrasts with wt, where H50 wraps around to participate in the Cu^{2+} coordination environment, in a structure incompatible with an α-helix.[2] H50Q may therefore enhance the stability of an extended α-helix. Moreover, H50Q in solution is less conformationally restricted than wt in the

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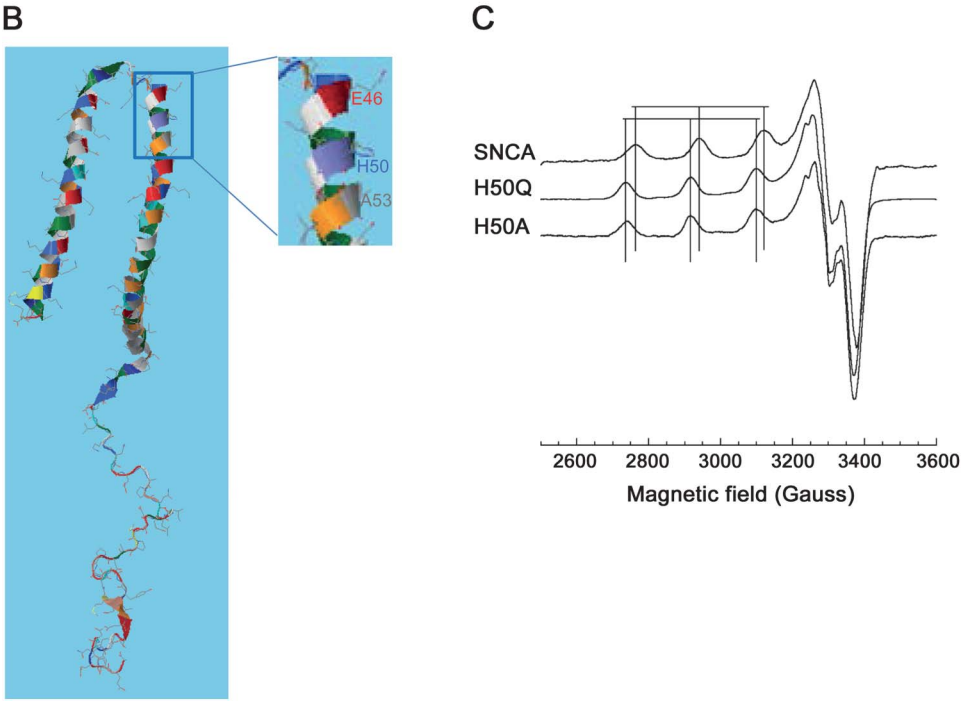
Figure 1 The H50Q mutation

A

Species	Protein ID	Partial sequence
Homo sapiens	P37840	KTKEGVV**H**GVATVAE
Mus musculus (mouse)	O55042	KTKEGVV**H**GVTTVAE
Rattus norvegicus (rat)	P37377	KTKEGVV**H**GVTTVAE
Bos taurus (cattle)	Q3T0G8	KTKEGVV**H**GVTTVAE
Serinus canaria (canary)	Q91448	RTKEGVV**H**GVTTVAE
Gallus gallus (chicken)	Q9I9H1	RTKEGVV**H**GVTTVAE
Taeniopygia guttata (zebra finch)	Q4JHT6	RTKEGVV**H**GVTTVAE
Xenopus laevis (African clawed frog)	Q7SZ02	KTKEGVV**H**GVTTVAE
Loxodonta africana (African elephant)	C6K8P9	KTKEGVV**H**GVTTVAE
Balaenoptera acutorostrata (mink whale)	C6K8P8	KTKEGVV**H**GVTTVAE
Phocoenoides phocoena (harbor porpoise)	C6K8Q0	KTKEGVV**H**GVTTVAE
Anolis carolinensis (American chameleon)	G1KNM6	KTRDGVV**Q**GVTTVAE
T.nigroviridis (Spotted green pufferfish)	H3C635	KTKD GVT**V**AV - - - AG



(A) Multiple alignment of α-synuclein (SNCA) indicates conservation of H50 (in bold) in higher vertebrates. Other nonconserved residues in different species in the region shown are underlined. (B) Schematic representation of SNCA secondary structure, highlighting the positions of the new and known mutations in exon 3. (C) X-band electron paramagnetic resonance spectra of wild-type (wt) SNCA, H50Q, and H50A, with 1.0 Eq Cu^{2+}. Vertical lines correspond to parallel hyperfine features of both wt and mutant forms.

presence of Cu^{2+}. Copper is elevated in the CSF of patients with PD,[3] it enhances SNCA fibril formation[4] and aggregation in cell cultures,[5] and dopaminergic neurons are vulnerable to Cu^{2+} in rats[6] and *Drosophila*.[7]

Based on the conservation of H50, the absence of the mutation in controls, and the predicted and observed functional effects, we propose c.150T>G (p.H50Q) as a pathogenic mutation, possibly with reduced penetrance, although we cannot exclude the possibility that it is a rare nonpathogenic variant. In vivo work, and identification of this mutation in other cases, will help confirm pathogenicity. The relevance of Cu^{2+} to PD merits further study.

Note added in proof: The same mutation has been reported in a Canadian patient in abstract form (Appel-Cresswell et al., Mov Disord 2012;27:S597).

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