Olfaction in *Parkin* heterozygotes and compound heterozygotes

The CORE-PD study

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ABSTRACT

Background: While Parkinson disease (PD) is consistently associated with impaired olfaction, one study reported better olfaction among *Parkin* mutation carriers than noncarriers. Whether olfaction differs between *Parkin* mutation heterozygotes and carriers of 2 *Parkin* mutations (compound heterozygotes) is unknown.

Objective: To assess the relationship between *Parkin* genotype and olfaction in PD probands and their unaffected relatives.

Methods: We administered the University of Pennsylvania Smell Identification Test (UPSIT) to 44 probands in the Consortium on Risk for Early-Onset Parkinson Disease study with PD onset ≤50 years (10 *Parkin* mutation heterozygotes, 9 compound heterozygotes, 25 noncarriers) and 80 of their family members (18 heterozygotes, 2 compound heterozygotes, 60 noncarriers). In the probands, linear regression was used to assess the association between UPSIT score (outcome) and *Parkin* genotype (predictor), adjusting for covariates. Among family members without PD, we compared UPSIT performance in heterozygotes vs noncarriers using generalized estimating equations, adjusting for family membership, age, gender, and smoking.

Results: Among probands with PD, compound heterozygotes had higher UPSIT scores (31.9) than heterozygotes (20.1) or noncarriers (19.9) ($p < 0.001$). These differences persisted after adjustment for age, gender, disease duration, and smoking. Among relatives without PD, UPSIT performance was similar in heterozygotes (32.5) vs noncarriers (32.4), and better than in heterozygotes with PD ($p = 0.001$).

Conclusion: Olfaction is significantly reduced among *Parkin* mutation heterozygotes with PD but not among their heterozygous relatives without PD. Compound heterozygotes with PD have olfaction within the normal range. Further research is required to assess whether these findings reflect different neuropathology in *Parkin* mutation heterozygotes and compound heterozygotes. *Neurology*® 2011;76:319–326

GLOSSARY

AAO = age at onset; **CORE-PD** = Consortium on Risk for Early-Onset Parkinson Disease study; **EOPD** = early-onset Parkinson disease; **GEE** = generalized estimating equation; **MMSE** = Mini-Mental State Examination; **PD** = Parkinson disease; **UPSIT** = University of Pennsylvania Smell Identification Test.

Mutations in the *Parkin* gene (PARK2; OMIM 600116)[1,2] are the most common genetic risk factors for early-onset Parkinson disease (PD) (EOPD)[3-13]; however, the role of heterozygous *Parkin* mutations in the pathogenesis of PD remains controversial.[14-17] While a comprehensive study showed a similar frequency of heterozygous point mutations in PD cases and controls,[16] PET studies show reduced fluorodopa uptake in nigrostriatal terminals in the caudate and posterior putamen of both symptomatic and asymptomatic *Parkin* heterozygotes compared to controls, similar to the reduction found in sporadic PD.[18-20]

Impairment in olfactory function is one of the earliest manifestations of idiopathic PD and has been reported up to 4 years before motor manifestations.[21] While impaired olfaction is frequently associated with PD, olfactory identification was reported to be better among 22 patients with parkinsonism with one or more *Parkin* mutations when compared to noncarrier

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Table 1 Parkinson disease probands with *Parkin* mutations (n = 19): mutation type, demographics, disease characteristics, and UPSIT performance

						Parkin mutations			
	UPSIT score	Age, y	UPDRS-III score	Disease duration, y	History of smoking	Mutation	Location within functional protein domain	Mutation	Location within functional protein domain
Compound heterozygotes									
1	37	58	8	14	Never[a]	Cys212Tyr	Unknown	Arg275Trp	RING1
2	36	53	18	20	Never	Exon 3 deletion	Ubiquitin	Exon 5 deletion	Unknown
3	35	45	16	26	Past	Exon 3 40 bp deletion	Ubiquitin	Arg275Trp	RING1
4[b]	34	30	15	11	Never	255delA	Ubiquitin	Exon 3–4 deletion	Ubiquitin
5	33	39	20	15	Current	255delA	Ubiquitin	Arg275Trp	RING1
6	32	50	2	8	Never	Arg42Pro	Ubiquitin	Exon 3 deletion	Ubiquitin
7	32	54	25	47	Past	Arg275Trp	RING1	Exon 4 deletion	Ubiquitin
8	27	40	23	14	Never	Arg275Trp	RING1	Gly430Asp	RING2
9[b]	21	59	17	24	Never	Exon 7–8 duplication	RING1	Exon 10 deletion	Unknown
Heterozygotes									
10	34	46	14	13	Current	Arg42Pro	Ubiquitin		
11	29	48	21	9	Never	202–3delAG	Ubiquitin		
12	26	39	27	14	Never	Exon 8 deletion	Unknown		
13	24	38	18	24	Never	Exon 3 40 bp deletion	Ubiquitin		
14	21	57	15	8	Never	Arg275Trp	RING1		
15	19	58	22	14	Never	Arg42Pro	Ubiquitin		
16	19	59	22	21	Never	Arg275Trp	RING1		
17	12	36	23	11	Never	Arg275Trp	RING1		
18	11	51	19	22	Never	Arg275Trp	RING1		
19	6	54	26	5	Never	Exon 3 40 bp deletion	Ubiquitin		

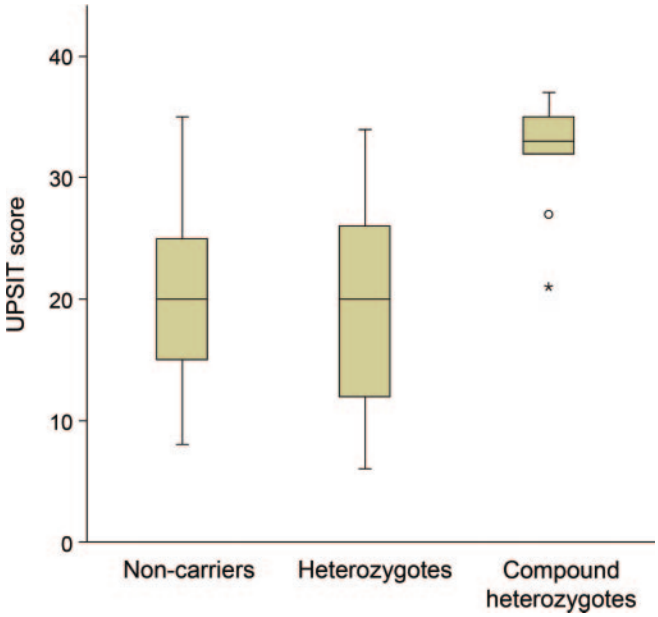
Abbreviations: UPDRS = Unified Parkinson's Disease Rating Scale; UPSIT = University of Pennsylvania Smell Identification Test.
[a] Never was defined as less than 100 cigarettes in a lifetime.
[b] These probands did not have sufficient family data to conclude that the 2 mutations are on 2 different alleles.

patients with PD.[22,23] Four of these carriers of *Parkin* mutations were heterozygotes (Dr. N. Khan, personal communication). Olfactory performance in *Parkin* mutation heterozygotes vs compound heterozygotes has never been reported. The purpose of this study was to compare olfactory function among carriers of a single *Parkin* mutation, carriers of 2 *Parkin* mutations, and noncarriers with and without PD.

METHODS **Participants.** This study included 44 individuals with EOPD (probands) who participated in Part II of the Consortium on Risk for Early-Onset Parkinson Disease study (CORE-PD), and 80 of their family members. The details of the CORE-PD study are described elsewhere.[24] In brief, patients with PD were recruited from 13 sites based on age at onset (AAO) of PD ≤50 years and performance on the Mini-Mental State Examination (MMSE)[25] >23 to ensure that a reliable history could be obtained from each subject. A blood sample for DNA was sent to the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org). In part II of CORE-PD, patients who carried *Parkin* mutations and a sample of those who did not carry *Parkin* mutations, as well as their family members, underwent a detailed neurologic, neuropsychological, and psychiatric assessment. Beginning in 2007, olfactory function was measured by the University of Pennsylvania Smell Identification Test (UPSIT,[26] Sensonics, Inc., Haddonfield, NJ). The UPSIT consists of 40 standardized encapsulated odors. Raw scores are calculated as the simple number of correct identifications ranging from 0 to 40; higher scores indicate better olfaction. Anosmia is defined as a score of 18 or lower and severe microsmia is defined as a score of 19–25 in people above age 15.[27] The reliability of UPSIT is well-established.[26] The UPSIT was obtained only if participants denied any upper respiratory illness, as suggested by the UPSIT manual. Smoking history was obtained on all participants. All examiners were unaware of the genetic status of the participants at the time of recruitment and thereafter.

Probands and family members were screened for *Parkin* mutations, as well as for common mutations in *LRRK2* and glucocere-

Figure 1 Box plot showing the University of Pennsylvania Smell Identification Test (UPSIT) scores in early-onset Parkinson disease participants who are *Parkin* compound heterozygotes, heterozygotes, or noncarriers



brosidase (*GBA*), using previously described methods,[24] after the examination was complete. In addition, most participants, including 31 of the 43 probands with PD and all family members, were screened for mutations in α-synuclein (*SNCA*; A157T, A88P, and E136K), DJ-1 (L166P, M26I, D149A, and A104T), and PTEN-induced putative kinase 1 (*PINK-1*; W437X and G309D).[28] None were aware of their mutation status. Carriers of *LRRK2* or *GBA* were excluded from the analyses. No carriers of mutations in *SNCA*, *DJ-1*, or *PINK-1* were detected.

Standard protocol approvals, registrations, and patient consents. Institutional review boards at all participating sites approved the protocols and consent procedures. Written informed consent was obtained from all participants in the study.

Data analysis. ***Probands.*** Demographics, clinical characteristics, and UPSIT performance were compared among the 3 groups defined by *Parkin* genotype using univariate analysis of variance and χ^2 tests as appropriate. A linear regression model was constructed to assess the association between UPSIT score (outcome) and mutation status (predictor), adjusting for age, gender, disease duration, and history of smoking (past and current vs never). Finally, we compared the proportion of probands with severe olfactory impairment (either severe microsmia or anosmia), defined as a score of 25 or lower (UPSIT manual)[27] among the genetic groups.

Unaffected family members. Given that only 2 family members without PD carried 2 *Parkin* mutations (both compound heterozygotes), we restricted the analysis of UPSIT performance in family members to *Parkin* mutation heterozygotes and noncarriers. We used generalized estimating equations (GEE) to adjust for familial clustering, age, gender, and history of smoking. Finally, we compared UPSIT performance between *Parkin* mutation heterozygotes with and without PD using the GEE model, to assess whether hyposmia was associated with PD among mutation carriers.

RESULTS

Probands with PD. Probands with PD included 9 carriers of 2 *Parkin* mutations (all compound heterozygotes), 10 *Parkin* mutation heterozygotes, and 25 noncarriers. The specific mutations in *Parkin* carriers are described in table 1. All the *Parkin* mutations reported are considered pathogenic.[24] Seven of the 9 carriers of 2 *Parkin* mutations had sufficient family data to conclude that the 2 *Parkin* mutations are on 2 different alleles (table 1).

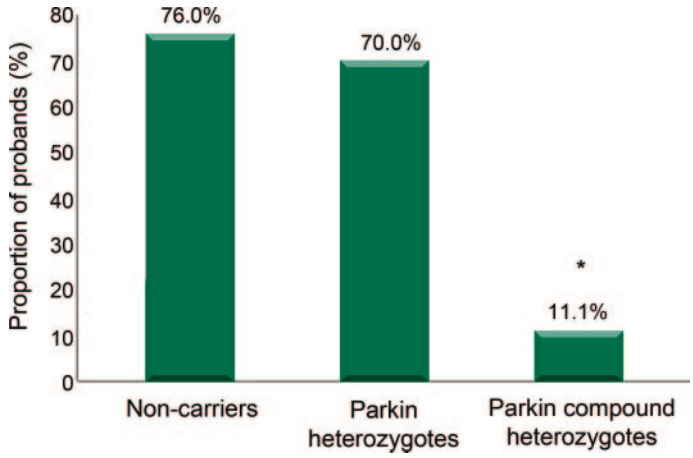
Probands with PD who carried 2 *Parkin* mutations performed significantly better on the UPSIT (31.9) than probands who were heterozygotes (20.1) or noncarriers (19.9), despite longer disease duration (figure 1, table 2). Mean UPSIT performance of probands who were *Parkin* mutation heterozygotes was within the range of severe microsmia (19–25), and was not significantly different from probands who were noncarriers. In a linear regression model including all probands with PD, carrying 2 *Parkin* mutations was associated with higher UPSIT scores when compared to *Parkin* mutation heterozygotes (10.9-point difference, $p = 0.005$) or to noncarriers (10.7-point difference, $p = 0.003$), after adjustment for gender, age, disease duration, and history of smoking. We did not find any association between olfaction and *Parkin* mutation type (point mutations vs gene dosage alterations), or with disease severity indi-

Table 2 UPSIT performance, demographics, and disease characteristics of noncarriers, *Parkin* heterozygotes, and *Parkin* compound heterozygotes

	Noncarriers (n = 25)	Heterozygotes (n = 10)	Carriers of 2 mutations (n = 9)	*p* Value
UPSIT score (SD, range)	19.9 (7.2, 8–35)	20.1 (8.6, 6–34)	31.9 (5.0, 21–37)	<0.001
Age, y (SD, range)	56 (8, 36–66)	49 (9, 36–59)	48 (10, 30–59)	0.015
Disease duration, y (SD, range)	13 (7, 1–27)	14 (6, 5–24)	20 (12, 8–47)	0.073
UPDRS-III (SD, range)	19 (6, 10–32)	21 (4, 14–27)	16 (7, 2–25)	0.295
Male sex (% male)	13 (52.0)	8 (80.0)	3 (33.3)	0.116
History of smoking	2 current, 6 past	1 current, 0 past	1 current, 2 past	0.319

Abbreviations: UPDRS = Unified Parkinson's Disease Rating Scale; UPSIT = University of Pennsylvania Smell Identification Test.

Figure 2 Proportion of probands with either anosmia or severe microsmia (University of Pennsylvania Smell Identification Test <26)



cators, including Unified Parkinson's Disease Rating Scale–III and disease duration. The proportion of *Parkin* mutation heterozygote and noncarrier probands who were either severely microsmic or anosmic combined (i.e., UPSIT score lower than 26) was higher than *Parkin* compound heterozygotes ($p = 0.02$, figure 2). The significant difference between *Parkin* mutation heterozygotes and compound heterozygotes did not change after excluding the 2 compound heterozygotes on whom there were insufficient family data to confirm compound heterozygocity status.

Family members without PD. Family members included 80 individuals from 37 families: 60 noncarriers, 18 *Parkin* mutation heterozygotes, and 2 compound heterozygotes. The specific mutations in family members who were *Parkin* carriers are described in table 3. A single heterozygote family member was diagnosed with PD and was excluded from the analysis (table 3). Among 79 family members without PD, the performance on the UPSIT of *Parkin* mutation heterozygotes (mean 32.5, range 18–39, table 3) was similar to that of noncarriers (mean 32.4, range 17–39, $p = 0.69$, adjusted for familial clustering, age, gender, and history of smoking in GEE model).

Table 3 Family members with *Parkin* mutations (n = 20): mutation type, demographics, and UPSIT performance

				Parkin mutations			
	UPSIT score	Age, y	History of smoking	Mutation	Location within functional protein domain	Mutation	Location within functional protein domain
Compound heterozygotes							
1	38	43	Current	202-3 deletion AG	Ubiquitin	Exon 3-4 deletion	Ubiquitin
2	37	48	Past	202-3 deletion AG	Ubiquitin	Exon 3-4 deletion	Ubiquitin
Heterozygotes							
3	39	60	Past	Exon 3 40 bp del	Ubiquitin		
4	38	29	Past	Arg275Trp	RING1		
5	37	45	Current	Exon 3 40 bp deletion	Ubiquitin		
6	37	85	Past	Cys212Tyr	Unknown		
7	35	24	Current	Arg275Trp	RING1		
8	35	28	Never	255 del A	Ubiquitin		
9	35	47	Never	Arg275Trp	RING1		
10	34	19	Never	Arg42Pro	Ubiquitin		
11	34	19	Never	Arg42Pro	Ubiquitin		
12	33	30	Never	Arg275Trp	RING1		
13	33	57	Never	Exon 3 40 bp deletion	Ubiquitin		
14	32	35	Never	255 del A	Ubiquitin		
15	32	41	Never	Arg275Trp	RING1		
16[a]	29	55	Never	Exon 5 deletion	Unknown		
17	28	37	Never	Arg275Trp	RING1		
18	27	73	Never	Exon 3 40 bp deletion	Ubiquitin		
19	25	21	Never	255 del A	Ubiquitin		
20	18	77	Never	Exon 3 deletion	Ubiquitin		

Abbreviation: UPSIT = University of Pennsylvania Smell Identification Test.
[a] This heterozygote carrier was diagnosed with Parkinson disease at age 41 and was excluded from the analysis.

Among individuals who were heterozygous carriers of *Parkin* mutations (probands with PD and relatives without PD), performance on the UPSIT was better in relatives without PD than in probands with EOPD (32.5 vs 20.1, $p = 0.001$, adjusted for familial clustering, age, gender, and history of smoking in a GEE model); however, in 12% (2/17) of the heterozygote family members without PD (from 2 different families) UPSIT performance was consistent with severe microsmia or anosmia. Both carried deletions in the *Parkin* gene (table 3). Both *Parkin* compound heterozygotes without PD had normal smell performance (table 3); however, statistical analysis was not performed given the small number (n = 2).

DISCUSSION Impairment in olfactory function is an early finding in idiopathic PD, and is not associated with severity of motor function or medication dosage.[29,30] Our study of olfaction in a genotyped sample of EOPD confirms previous reports of better olfaction in carriers of 2 *Parkin* mutations with PD,[22,23] and demonstrates impaired olfaction in *Parkin* mutation heterozygotes with PD, similar to that of people with PD who were *Parkin* noncarriers and significantly worse than compound heterozygotes with PD. The role of *Parkin* mutation heterozygosity in the pathogenesis of PD is controversial.[14-17] Data supporting the hypothesis that heterozygous mutations convey a risk for PD include imaging findings[18-20] and studies showing an increased frequency of heterozygous carriers in PD cases compared to controls.[15] Our finding of similar olfactory performance in *Parkin* heterozygotes with PD and noncarriers with PD may be viewed as consistent with the hypothesis that *Parkin* mutation heterozygosity is not an independent risk factor for PD. Alternatively, poor performance on the UPSIT may reflect a different distribution of pathology among *Parkin* mutation heterozygotes compared to compound heterozygotes.

Olfactory impairment in PD is associated with Lewy body infiltration of the olfactory bulb and tract.[31] Neuropathologic staging of PD suggests the presence of Lewy bodies in the olfactory bulb in Braak stages 1–2, even before the disease reaches the substantia nigra (stage 3).[32] To our knowledge, 7 autopsies of individuals with *Parkin* mutations have been reported. Only 2 of the 6 homozygotes/compound heterozygotes had Lewy bodies,[33,34] whereas the clinical course and pathology from a single autopsy of a *Parkin* mutation heterozygote was consistent with progressive supranuclear palsy.[35] Based on our findings of different olfactory performance in *Parkin* mutation heterozygotes and *Parkin* compound heterozygotes, we hypothesize that better UPSIT performance is inversely correlated with Lewy body pathology, and that *Parkin* heterozygotes with PD have Lewy bodies in the olfactory bulb; however, autopsy data are lacking, and further research is required to address the relationship between olfactory performance and the underlying disease mechanism.

Limitations of our study include its sample size, which did not allow us to analyze point mutation carriers and gene dosage alterations carriers in the *Parkin* gene separately. While we excluded carriers of common mutations in *LRRK2* and *GBA* to avoid confounders, we only screened for specific mutations and did not sequence the α-synuclein *DJ-1* or *PINK-1* genes, which are associated with impaired olfaction.[36]

When *Parkin* mutation heterozygotes with and without PD were compared, olfactory impairment was associated with PD, supporting the notion that hyposmia in *Parkin* mutation heterozygotes is related to PD rather than to *Parkin* genotype. However, we also noted that 2 (12%) of the *Parkin* mutation heterozygotes without PD manifested impaired olfaction. In light of 2 prospective studies that demonstrated that 10%–13% of individuals who had both hyposmia and abnormal functional imaging at baseline developed PD over a 2- to 5-year period,[37,38] a longitudinal follow-up of these carriers is warranted to determine whether hyposmia can be used as an early marker of PD in *Parkin* heterozygous mutation carriers, and whether Parkin mutation heterozygosity is indeed a risk factor for PD.

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Dr. Pfeiffer serves on a scientific advisory board for the National Parkinson Foundation; serves on the editorial board of *Parkinsonism and Related Disorders*; receives publishing royalties for *Parkinson's Disease* (Taylor & Francis, 2008), *Parkinson's Disease and Nonmotor Dysfunction* (Humana, 2008), and *Neuro-Gastroenterology* (Butterworth-Heinemann, 2008); serves as a consultant for Solvay Pharmaceuticals, Inc., Theravance, Inc., Genactis, Inc., and Schlesinger Associates; serves on speakers' bureaus for and has received speaker honoraria from Boehringer Ingelheim, Novartis, and Teva Pharmaceutical Industries Ltd.; receives research support from Novartis, Boehringer Ingelheim, UCB/ SCHWARZ PHARMA, Santhera Pharmaceuticals, and Molecular Biometrics, Inc., Columbia University, Weill Cornell Medical College, Northwestern University, Indiana University, Parkinson Study Group, and the Michael J. Fox Foundation; and has served as a consultant in medico-legal cases. 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REFERENCES

1. Kitada T, Asakawa S, Hattori N, et al. Mutations in the *Parkin* gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608.
2. Hedrich K, Eskelson C, Wilmot B, et al. Distribution, type, and origin of *Parkin* mutations: review and case studies. Mov Disord 2004;19:1146–1157.
3. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the *Parkin* gene: French Parkinson's Disease Genetics Study Group. N Engl J Med 2000;342:1560–1567.
4. Hedrich K, Marder K, Harris J, et al. Evaluation of 50 probands with early-onset Parkinson's disease for *Parkin* mutations. Neurology 2002;58:1239–1246.
5. Abbas N, Lucking CB, Ricard S, et al. A wide variety of mutations in the *Parkin* gene are responsible for autosomal recessive parkinsonism in Europe: French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. Hum Mol Genet 1999;8:567–574.
6. Periquet M, Latouche M, Lohmann E, et al. *Parkin* mutations are frequent in patients with isolated early-onset parkinsonism. Brain 2003;126:1271–1278.
7. Lohmann E, Periquet M, Bonifati V, et al. How much phenotypic variation can be attributed to *Parkin* genotype? Ann Neurol 2003;54:176–185.
8. Camargos ST, Dornas LO, Momeni P, et al. Familial parkinsonism and early onset Parkinson's disease in a Brazilian movement disorders clinic: phenotypic characterization and frequency of SNCA, PRKN, PINK1, and LRRK2 mutations. Mov Disord 2009;24:662–666.
9. Macedo MG, Verbaan D, Fang Y, et al. Genotypic and phenotypic characteristics of Dutch patients with early onset Parkinson's disease. Mov Disord 2009;24:196–203.
10. Hertz JM, Ostergaard K, Juncker I, et al. Low frequency of *Parkin*, tyrosine hydroxylase, and GTP cyclohydrolase I gene mutations in a Danish population of early-onset Parkinson's disease. Eur J Neurol 2006;13:385–390.
11. Chung EJ, Ki CS, Lee WY, Kim IS, Kim JY. Clinical features and gene analysis in Korean patients with early-onset Parkinson disease. Arch Neurol 2006;63:1170–1174.
12. Bras J, Guerreiro R, Ribeiro M, et al. Analysis of Parkinson disease patients from Portugal for mutations in SNCA, PRKN, PINK1 and LRRK2. BMC Neurol 2008;8:1.
13. Vinish M, Prabhakar S, Khullar M, Verma I, Anand A. Genetic screening reveals high frequency of PARK2 mutations and reduced *Parkin* expression conferring risk for Parkinsonism in North West India. J Neurol Neurosurg Psychiatry 2010;81:166–170.
14. Klein C, Lohmann-Hedrich K. Impact of recent genetic findings in Parkinson's disease. Curr Opin Neurol 2007;20:453–464.
15. Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE. Deciphering the role of heterozygous mutations in genes associated with parkinsonism. Lancet Neurol 2007;6:652–662.
16. Kay DM, Moran D, Moses L, et al. Heterozygous *Parkin* point mutations are as common in control subjects as in Parkinson's patients. Ann Neurol 2007;61:47–54.
17. Pankratz N, Kissell DK, Pauciulo MW, et al. *Parkin* dosage mutations have greater pathogenicity in familial PD than simple sequence mutations. Neurology 2009;73:279–286.
18. Hilker R, Klein C, Ghaemi M, et al. Positron emission tomographic analysis of the nigrostriatal dopaminergic system in familial parkinsonism associated with mutations in the *Parkin* gene. Ann Neurol 2001;49:367–376.
19. Portman AT, Giladi N, Leenders KL, et al. The nigrostriatal dopaminergic system in familial early onset parkinsonism with *Parkin* mutations. Neurology 2001;56:1759–1762.
20. Scherfler C, Khan NL, Pavese N, et al. Striatal and cortical pre- and postsynaptic dopaminergic dysfunction in sporadic *Parkin*-linked parkinsonism. Brain 2004;127:1332–1342.
21. Ross GW, Petrovitch H, Abbott RD, et al. Association of olfactory dysfunction with risk for future Parkinson's disease. Ann Neurol 2008;63:167–173.
22. Khan NL, Katzenschlager R, Watt H, et al. Olfaction differentiates *Parkin* disease from early-onset parkinsonism and Parkinson disease. Neurology 2004;62:1224–1226.
23. Verbaan D, Boesveldt S, van Rooden SM, et al. Is olfactory impairment in Parkinson disease related to phenotypic or genotypic characteristics? Neurology 2008;71:1877–1882.
24. Marder KS, Tang MX, Mejia-Santana H, et al. Predictors of *Parkin* mutations in early-onset Parkinson disease: the consortium on risk for early-onset Parkinson disease study. Arch Neurol 2010;67:731–738.
25. Folstein MF, Folstein SE, McHugh PRP. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
26. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol Behav 1984;32:489–502.
27. Doty R. The Smell Identification Test Administration Manual. Haddon Heights, NJ: Sensonics, Inc.; 1995.
28. Alcalay RN, Caccappolo E, Mejia-Santana H, et al. Frequency of known mutations in early onset PD: implication for genetic counseling: the CORE-PD study. Arch Neurol (in press 2010).
29. Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988;38:1237–1244.
30. Doty RL, Riklan M, Deems DA, Reynolds C, Stellar S. The olfactory and cognitive deficits of Parkinson's disease: evidence for independence. Ann Neurol 1989;25:166–171.
31. Hawkes C. Olfaction in neurodegenerative disorder. Mov Disord 2003;18:364–372.
32. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rub U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 2002;249(suppl 3):III/1–5.
33. Farrer M, Chan P, Chen R, et al. Lewy bodies and parkinsonism in families with *Parkin* mutations. Ann Neurol 2001;50:293–300.
34. Pramstaller PP, Schlossmacher MG, Jacques TS, et al. Lewy body Parkinson's disease in a large pedigree with 77 *Parkin* mutation carriers. Ann Neurol 2005;58:411–422.

35. Morales B, Martinez A, Gonzalo I, et al. Steele-Richardson-Olszewski syndrome in a patient with a single C212Y mutation in the *Parkin* protein. Mov Disord 2002; 17:1374–1380.
36. Ferraris A, Ialongo T, Passali GC, et al. Olfactory dysfunction in Parkinsonism caused by PINK1 mutations. Mov Disord 2009;24:2350–2357.
37. Ponsen MM, Stoffers D, Booij J, van Eck-Smit BL, Wolters E, Berendse HW. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Ann Neurol 2004;56:173–181.
38. Ponsen MM, Stoffers D, Wolters E, Booij J, Berendse HW. Olfactory testing combined with dopamine transporter imaging as a method to detect prodromal Parkinson's disease. J Neurol Neurosurg Psychiatry 2010;81:396–399.

Editor's Note to Authors and Readers: Levels of Evidence coming to *Neurology*®

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*® that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the classification scheme requirements shown below (left). While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care, as shown below (right). For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.[1-3]

REFERENCES

1. French J, Gronseth G. Lost in a jungle of evidence: we need a compass. Neurology 2008;71:1634–1638.
2. Gronseth G, French J. Practice parameters and technology assessments: what they are, what they are not, and why you should care. Neurology 2008;71:1639–1643.
3. Gross RA, Johnston KC. Levels of evidence: taking *Neurology*® to the next level. Neurology 2009;72:8–10.

Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class II. A randomized, controlled clinical trial of the intervention of interest in a representative population with masked or objective outcome assessment that lacks one criterion a-e in Class I or a prospective matched cohort study with masked or objective outcome assessment in a representative population that meets b-e in Class I. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

AAN classification of recommendations

A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies.)

B = Probably effective, ineffective, or harmful (or probably useful/predictive or not useful/predictive) for the given condition in the specified population. (Level B rating requires at least one Class I study or two consistent Class II studies.)

C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.