ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Ювенилна Паркинсонова болест (PARK2; PARK6; PARK7; PARK23; PARK 12; PARK 3; PARK 10; PARK 13; PARK5)
Определение на заболяването
Ювенилна Паркинсонова болест е форма на Паркинсоновата болест, характеризираща се с възраст на начало между 21 и 45 години. Предоминантно в началото се развиват ригидност и болезнени крампи, последвани от тремор, брадикинезия, дистония, нарушения в походката с падания и други немоторни симптоми. За повечето форми на заболяването е характерна също бавна прогресия на заболяването и изразен отговор на допаминергична терапия.
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G20
Код на заболяването по Orpha code
ORPHA2828
Епидемиологични данни за заболяването в Република България
Предполага се 1-5 / 10 000, съответстващо на това в останалите страни в Европейски съюз
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Milanov I, Kmetska K, Karakolev B, Nedialkov E. Prevalence of Parkinson's disease in Bulgaria. Neuroepidemiology. 2001;20(3):212-4. 2. Farlow J., Pankratz ND, Wojcieszek J., Foroud T. Parkinson Disease Overview GeneReviews® Last Update: February 27, 2014.
Епидемиологични данни за заболяването в Европейския съюз
1-5 / 10 000; Разпространението на Ювенилна Паркинсонова болест в Европейския съюз се оценява на 1/8,000-1/5,000 (5-10 % от всички пациенти с ПБ). Мъжете са по-засегнати от жените (1.7:1). Наблюдава се, че жените развиват заболяването средно около 2 години по-късно от мъжете.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Wang, Y., Clark, L. N., Louis, E. D., Mejia-Santana, H., Harris, J., Cote, L. J., Waters, C., Andrews, H., Ford, B., Frucht, S., Fahn, S., Ottman, R., Rabinowitz, D., Marder, K. Risk of Parkinson disease in carriers of parkin mutations: estimation using the kin-cohort method. Arch. Neurol. 65: 467-474, 2008. 2. Farlow J., Pankratz ND, Wojcieszek J., Foroud T. Parkinson Disease Overview GeneReviews® Last Update: February 27, 2014.
Оценка на съответствието на заболяването с дефиницията за рядко заболяване

съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето
Заболяването е с разпространение до 5/ 10 000 души от населението на Европейския съюз.
Критерии за диагностициране на заболяването
Диагностициране на заболяването (дефиниция на случай): Признаците и симптомите на заболяването: Ювенилна Паркинсонова болест е с възраст на начало между 21 и 45 години. Предоминантно в началото се развиват ригидност и болезнени крампи, последвани от тремор, брадикинезия, нарушения в походката с падания. В сравнение с класическата ПБ се наблюдава нисък риск за развитие на падания и фрийзинг феномени, но по-висок риск за дистония, моторни флуктуации и леводопа индуцирани дискинезии. При Паркинсонова болест с ранно начало се съобщава за преобладаване на немоторните симптоми включително апатия, генерализирана тревожност, панически заболявания, социална фобия, депресия, психоза (халюцинации), поведенчески нарушения (агитираност и заболявания свързани с контрола на импулсивността), деменция и когнитивни нарушения в сравнение с класическата спорадична форма на заболяването. Жените е по-вероятно да се представят с тремор или да развият апатия, тревожност, депресия или леводопа-индуцирани дискинезии. По същия начин нелекуваните пациенти с Паркинсонова болест с ранно начало се представят много по-често с крампи и дистонна поза в сравнение с по-възрастните пациенти с ПБ. Типични черти на PARK2 са диурна флуктуациите с подобрение след сън, дистония на стъпало, хиперрефлексия и ранни леводопа-индуцирани дискинезии. Етиологията и патогенезата: Точната етиология на Паркинсонова болест с ранно начало е все още неизяснена. Симптомите на Паркинсонова болест с ранно начало се смятат да са в резултат на дегенерация на допа-продуциращите неврони на субстанция нигра в резултат на инфекциозно заболяване, фармакотерапия или генетични фактори. Мутации в PARK2 (6q25.2-q27), PARK6 (PINK1, мутация в хромозома 1p36.12) и PARK7 (1p36.23) гените са въвлечени в някои случаи на Паркинсонова болест с ранно начало. Shimura и колеги (2001) предполагат, че alpha-synuclein и parkin взаимодействат функционално, а именно че parkin ubiquitinates alpha-synuclein нормално и че този процес е променен при автозомно-рецесивната ПБ. Shimura и колеги (2001) идентифицират протеинов комплекс в нормалния човешки мозък, който включва parkin като E3 ubiquitin ligase, UBCH7 (като той е свързан с E2 ubiquitin-conjugating ензим) и една нова 22-kD глюкозилатна форма на alpha-synuclein (alpha-Sp22) като субстрат. Обратно на нормалния parkin, при автозомно-рецесивните форми на ПБ мутиралият parkin не успява да се върже с alpha-Sp22. Shimura и колеги (2001) заключават че alpha-Sp22 е субстрата за parkin ubiquitin ligase активност при нормални условия и че загубата на parkin функцията причинява патологично натрупване на alpha-Sp22. Mortiboys и колеги (2008) установяват, че фибробластите от ПБ пациенти с биалелни мутации на PARK2 гена имат значително намален митохондреален комплекс I активност и АТФ продукция в сравнение с контроли. Фибробластите на пациентите показват също промяна в морфологията, което включва по-голяма степен на митохондриално разклоняване, както и увеличена чувствителност към митохондриални токсини.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Rudolph, A., Shults, C.,

Conneally, P. M., Foroud, T., the Parkinson Study Group. Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. Am. J. Hum. Genet. 71: 124-135, 2002.

2. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Murrell, J., Rudolph, A., Shults, C. W., Conneally, P. M., Foroud, T., Parkinson Study Group. Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum. Molec. Genet. 12: 2599-2608, 2003.
3. Hicks, A. A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H. S., Sainz, J., Frigge, M. L., Kong, A., Gulcher, J. R., Stefansson, K., Sveinbjornsdottir, S. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann. Neurol. 52: 549-555, 2002.
4. Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Koller, W. C., Lyons, K., Pahwa, R., Stern, M. B., Colcher, A., Hiner, B. C., Jankovic, J., and 20 others. Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 286: 2239-2244, 2001.
5. Takahashi, H., Ohama, E., Suzuki, S., Horikawa, Y., Ishikawa, A., Morita, T., Tsuji, S., Ikuta, F. Familial juvenile parkinsonism: clinical and pathologic study in a family. Neurology 44: 437-441, 1994.
6. Ishikawa, A., Tsuji, S. Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. Neurology 47: 160-166, 1996.
7. Bonifati, V., Vanacore, N., Meco, G. Anticipation of onset age in familial Parkinson's disease. Neurology 44: 1978-1979, 1994.
8. Mitsui, T., Kawai, H., Sakoda, S., Miyata, M., Saito, S. Hereditary parkinsonism with multiple system degeneration: beneficial effect of anticholinergics, but not of levodopa. J. Neurol. Sci. 125: 153-157, 1994.
9. Wang, Y., Clark, L. N., Louis, E. D., Mejia-Santana, H., Harris, J., Cote, L. J., Waters, C., Andrews, H., Ford, B., Frucht, S., Fahn, S., Ottman, R., Rabinowitz, D., Marder, K. Risk of Parkinson disease in carriers of parkin mutations: estimation using the kin-cohort method. Arch. Neurol. 65: 467-474, 2008.
10. Klein, C., Pramstaller, P. P., Kis, B., Page, C. C., Kann, M., Leung, J., Woodward, H., Castellan, C. C., Scherer, M., Vieregge, P., Breakefield, X. O., Kramer, P. L., Ozelius, L. J. Parkin deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. Ann. Neurol. 48: 65-71, 2000.
11. Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S., Selkoe, D. J. Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. Science 293: 263-269, 2001.
12. Mortiboys, H., Thomas, K. J., Koopman, W. J. H., Klaffke, S., Abou-Sleiman, P., Olpin, S., Wood, N. W., Willems, P. H. G. M., Smeitink, J. A. M., Cookson, M. R., Bandmann, O. Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. Ann. Neurol. 64: 555-565, 2008.
13. DeStefano, A. L., Lew, M. F., Golbe, L. I., Mark, M. H., Lazzarini, A. M., Guttman, M., Montgomery, E., Waters, C. H., Singer, C., Watts, R. L., Currie, L. J., Wooten, G. F., and 19 others. PARK3 influences age at onset in Parkinson disease: a genome scan in the GenePD study. Am. J. Hum. Genet. 70: 1089-1095, 2002.
14. Hatano, Y., Sato, K., Elibol, B., Yoshino, H., Yamamura, Y., Bonifati, V., Shinotoh, H., Asahina, M., Kobayashi, S., Ng, A. R., Rosales, R. L., Hassin-Baer, S., and 9

others. PARK6-linked autosomal recessive early-onset parkinsonism in Asian populations. Neurology 63: 1482-1485, 2004.
15. Bonifati, V., Rohe, C. F., Breedveld, G. J., Fabrizio, E., De Mari, M., Tassorelli, C., Tavella, A., Marconi, R., Nicholl, D. J., Chien, H. F., Fincati, E., Abbruzzese, G. {and 24 others}: Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. Neurology 65: 87-95, 2005.
16. Albanese, A., Valente, E. M., Romito, L. M., Bellacchio, E., Elia, A. E., Dallapiccola, B. The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. Neurology 64: 1958-1960, 2005.
17. van Duijn, C. M., Dekker, M. C. J., Bonifati, V., Galjaard, R. J., Houwing-Duistermaat, J. J., Snijders, P. J. L. M., Testers, L., Breedveld, G. J., Horstink, M., Sandkuijl, L. A., van Swieten, J. C., Oostra, B. A., Heutink, P. PARK7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am. J. Hum. Genet. 69: 629-634, 2001.
18. Abou-Sleiman, P. M., Healy, D. G., Quinn, N., Lees, A. J., Wood, N. W. The role of pathogenic DJ-1 mutations in Parkinson's disease. Ann. Neurol. 54: 283-286, 2003.
19. Strauss, K. M., Martins, L. M., Plun-Favreau, H., Marx, F. P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Muller, T., Bornemann, A., Wolburg, H., Downward, J., Riess, O., Schulz, J. B., Kruger, R. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum. Molec. Genet. 14: 2099-2111, 2005.
20. Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D., Polymeropoulos, M. H. The ubiquitin pathway in Parkinson's disease. (Letter) Nature 395: 451-452, 1998.
21. Lesage, S., Drouet, V., Majounie, E., Deramecourt, V., Jacoupy, M., Nicolas, A., Cormier-Dequaire, F., Hassoun, S. M., Pujol, C., Ciura, S., Erpapazoglou, Z., Usenko, T., and 32 others. Loss of VPS13C function in autosomal-recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. Am. J. Hum. Genet. 98: 500-513, 2016.

Алгоритми за диагностициране на заболяването

Алгоритми за диагностициране на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест.

Анамнезата: Характерно за клиничните изяви наличието на тремор се наблюдава в 85% от пациентите с Паркинсонова болест с ранно начало, ранната възраст на начало на заболяването, фамилната анамнеза ПБ и добрия отговор на допаминергична терапия.

Диференциалната диагноза на заболяването: Атипичен ювенилен паркинсонизъм; Паркинсонова болест с късно начало; Наследствен есенциален тремор, болест на Wilson, болест на Gaucher тип 3, Pantothenate kinase-асоциирана, ювенилна болест на Хънтингтон и лезии на базални ганглии.

Лабораторни, образни и хистологични изследвания: PET/SPECT изследванията показват намалено натрупване на допаминовите транспортери в базалните ганглии. Финалната диагноза се поставя на базата на наличието на телца на lewy в мозъка при аутопсия. Ohsawa и колеги (2005) предполагат, че редукцията на SNAP амплитудата при ПБ пациенти под 60 години би могла да е индикатор за PARK2 мутации, като заключават, че сензорната аксонална невропатия е честа черта на заболяването.

Генетични изследвания и медико-генетично консултиране: В повечето случаи Паркинсоновата болест с ранно начало е спорадична. При все това се наблюдават и фамилни случаи с мутации в PARK2 (хомозиготна или комбинирана хетерозиготна мутация в parkin гена на хромозома 6q26), PARK6 (PINK1), PARK7 (хомозиготна или комбинирана хетерозиготна мутация на DJ1 ген на хромозома 1p36) и PARK23 (хомозиготна или комбинирана хетерозиготна мутация на VPS13C ген 15q22), при които се предполага автозомно-рецесивно унаследяване, както и PARK 12 (свързан с X хромозомата), PARK 3 (хромозома 2p13), PARK 10 (хромозома 1p32), PARK 13 (хетерозиготна мутация на HTRA2 ген на хромозома 2p13), PARK5 (хетерозиготна мутация на UCHL1 ген на хромозома 4p13) .

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Rudolph, A., Shults, C., Conneally, P. M., Foroud, T., the Parkinson Study Group. Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. Am. J. Hum. Genet. 71: 124-135, 2002.
2. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Murrell, J., Rudolph, A., Shults, C. W., Conneally, P. M., Foroud, T., Parkinson Study Group. Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum. Molec. Genet. 12: 2599-2608, 2003.
3. Hicks, A. A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H. S., Sainz, J., Frigge, M. L., Kong, A., Gulcher, J. R., Stefansson, K., Sveinbjornsdottir, S. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann. Neurol. 52: 549-555, 2002.
4. Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Koller, W. C., Lyons, K., Pahwa, R., Stern, M. B., Colcher, A., Hiner, B. C., Jankovic, J., and 20 others. Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 286: 2239-2244, 2001.
5. Takahashi, H., Ohama, E., Suzuki, S., Horikawa, Y., Ishikawa, A., Morita, T., Tsuji, S., Ikuta, F. Familial juvenile parkinsonism: clinical and pathologic study in a family. Neurology 44: 437-441, 1994.
6. Ishikawa, A., Tsuji, S. Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. Neurology 47: 160-166, 1996.
7. Bonifati, V., Vanacore, N., Meco, G. Anticipation of onset age in familial Parkinson's disease. Neurology 44: 1978-1979, 1994.
8. Mitsui, T., Kawai, H., Sakoda, S., Miyata, M., Saito, S. Hereditary parkinsonism with multiple system degeneration: beneficial effect of anticholinergics, but not of levodopa. J. Neurol. Sci. 125: 153-157, 1994.
9. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Rudolph, A., Shults, C., Conneally, P. M., Foroud, T., the Parkinson Study Group. Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. Am. J. Hum. Genet. 71: 124-135, 2002.
10. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Murrell, J., Rudolph, A., Shults, C. W., Conneally, P. M., Foroud, T., Parkinson Study Group. Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum. Molec. Genet. 12: 2599-2608, 2003.

11. Hicks, A. A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H. S., Sainz, J., Frigge, M. L., Kong, A., Gulcher, J. R., Stefansson, K., Sveinbjornsdottir, S. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann. Neurol. 52: 549-555, 2002.
12. Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Koller, W. C., Lyons, K., Pahwa, R., Stern, M. B., Colcher, A., Hiner, B. C., Jankovic, J., and 20 others. Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 286: 2239-2244, 2001.
13. Takahashi, H., Ohama, E., Suzuki, S., Horikawa, Y., Ishikawa, A., Morita, T., Tsuji, S., Ikuta, F. Familial juvenile parkinsonism: clinical and pathologic study in a family. Neurology 44: 437-441, 1994.
14. Ishikawa, A., Tsuji, S. Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. Neurology 47: 160-166, 1996.
15. Bonifati, V., Vanacore, N., Meco, G. Anticipation of onset age in familial Parkinson's disease. Neurology 44: 1978-1979, 1994.
16. Mitsui, T., Kawai, H., Sakoda, S., Miyata, M., Saito, S. Hereditary parkinsonism with multiple system degeneration: beneficial effect of anticholinergics, but not of levodopa. J. Neurol. Sci. 125: 153-157, 1994.
17. Portman, A. T., Giladi, N., Leenders, K. L., Maguire, P., Veenma-van der Duin, L., Swart, J., Pruim, J., Simon, E. S., Hassin-Baer, S., Korczyn, A. D. The nigrostriatal dopaminergic system in familial early onset parkinsonism with parkin mutations. Neurology 56: 1759-1762, 2001.
18. Wang, Y., Clark, L. N., Louis, E. D., Mejia-Santana, H., Harris, J., Cote, L. J., Waters, C., Andrews, H., Ford, B., Frucht, S., Fahn, S., Ottman, R., Rabinowitz, D., Marder, K. Risk of Parkinson disease in carriers of parkin mutations: estimation using the kin-cohort method. Arch. Neurol. 65: 467-474, 2008.
19. DeStefano, A. L., Lew, M. F., Golbe, L. I., Mark, M. H., Lazzarini, A. M., Guttman, M., Montgomery, E., Waters, C. H., Singer, C., Watts, R. L., Currie, L. J., Wooten, G. F., and 19 others. PARK3 influences age at onset in Parkinson disease: a genome scan in the GenePD study. Am. J. Hum. Genet. 70: 1089-1095, 2002.
20. Hatano, Y., Sato, K., Elibol, B., Yoshino, H., Yamamura, Y., Bonifati, V., Shinotoh, H., Asahina, M., Kobayashi, S., Ng, A. R., Rosales, R. L., Hassin-Baer, S., and 9 others. PARK6-linked autosomal recessive early-onset parkinsonism in Asian populations. Neurology 63: 1482-1485, 2004.
21. Bonifati, V., Rohe, C. F., Breedveld, G. J., Fabrizio, E., De Mari, M., Tassorelli, C., Tavella, A., Marconi, R., Nicholl, D. J., Chien, H. F., Fincati, E., Abbruzzese, G. {and 24 others}: Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. Neurology 65: 87-95, 2005.
22. van Duijn, C. M., Dekker, M. C. J., Bonifati, V., Galjaard, R. J., Houwing-Duistermaat, J. J., Snijders, P. J. L. M., Testers, L., Breedveld, G. J., Horstink, M., Sandkuijl, L. A., van Swieten, J. C., Oostra, B. A., Heutink, P. PARK7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am. J. Hum. Genet. 69: 629-634, 2001.
23. Abou-Sleiman, P. M., Healy, D. G., Quinn, N., Lees, A. J., Wood, N. W. The role of pathogenic DJ-1 mutations in Parkinson's disease. Ann. Neurol. 54: 283-286, 2003.
24. Hicks, A. A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H. S., Sainz, J., Frigge, M. L., Kong, A., Gulcher, J. R., Stefansson, K., Sveinbjornsdottir, S. A

susceptibility gene for late-onset idiopathic Parkinson's disease. Ann. Neurol. 52: 549-555, 2002.
25. Strauss, K. M., Martins, L. M., Plun-Favreau, H., Marx, F. P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Muller, T., Bornemann, A., Wolburg, H., Downward, J., Riess, O., Schulz, J. B., Kruger, R. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum. Molec. Genet. 14: 2099-2111, 2005.
26. Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D., Polymeropoulos, M. H. The ubiquitin pathway in Parkinson's disease. (Letter) Nature 395: 451-452, 1998.
27. Lesage, S., Drouet, V., Majounie, E., Deramecourt, V., Jacoupy, M., Nicolas, A., Cormier-Dequaire, F., Hassoun, S. M., Pujol, C., Ciura, S., Erpapazoglou, Z., Usenko, T., and 32 others. Loss of VPS13C function in autosomal-recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. Am. J. Hum. Genet. 98: 500-513, 2016.

Алгоритми за лечение на заболяването

Алгоритми за лечение на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, а на когнитивните и други немоторни симптоми съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.

Алгоритми за проследяване на заболяването

Алгоритми за проследяване на заболяването (Необходимостта от последващи болнични и извънболнични грижи; Необходимостта от консултации с други специалисти): Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Прогнозата на заболяването: Средна преживяемост след началото на заболяването е около 30 години. Паркинсонова болест с ранно начало се свързва с бавна прогресия на заболяването и по-леко когнитивно влошаване в началото. При все това пациентите с Паркинсонова болест с ранно начало е по-вероятно да имат усложнения като тежки изразени инвалидизиращи дискинезии, болезнена дистония, непредсказуеми и тежки моторни флуктуации. По-лошо качество на живот се отбелязва в резултат на социални и психомоторни нарушения..

Възможни усложнения, честота и тежест на усложненията и др.: След 5-годишна Леводопа терапия 30-40% от пациентите (59-100% до 10-тата година) развиват дискинезии и моторни флуктуации.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.

2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
3. Lohmann, E., Periquet, M., Bonifati, V., Wood, N. W., De Michele, G., Bonnet, A.-M., Fraix, V., Broussolle, E., Horstink, M. W. I. M., Vidailhet, M., Verpillat, P., Gasser, T., French Parkinson's Disease Genetics Study Group, European Consortium on Genetic Susceptibility in Parkinson's Disease, and 11 others. How much phenotypic variation can be attributed to parkin genotype? Ann. Neurol. 54: 176-185, 2003.
4. Hatano, Y., Sato, K., Elibol, B., Yoshino, H., Yamamura, Y., Bonifati, V., Shinotoh, H., Asahina, M., Kobayashi, S., Ng, A. R., Rosales, R. L., Hassin-Baer, S., and 9 others. PARK6-linked autosomal recessive early-onset parkinsonism in Asian populations. Neurology 63: 1482-1485, 2004.
5. Albanese, A., Valente, E. M., Romito, L. M., Bellacchio, E., Elia, A. E., Dallapiccola, B. The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. Neurology 64: 1958-1960, 2005.

Алгоритми за рехабилитация на заболяването

Алгоритми за рехабилитация на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.

Необходими дейности за профилактика на заболяването (ако такива са приложими)

Дейности за профилактика на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.

Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба

Създаването на Национален експертен център „Редки невродегенеративни заболявания, протичащи с когнитивни, поведенчески и моторни нарушения" за диагностика, лечение и проследяване и рехабилитация включително и на пациенти с това заболявания под ръководството на чл.кор.проф.д-р Л. Трайков, дмн (национален експерт с най-голям опит и принос за диагностиката и лечението на тези заболявания).

Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)

Опитът на кандидатстващия експертен център под ръководството на чл. кор. проф.Трайков за диагноза и лечение на редки заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, датира от 2001 година със създаването на център за диагноза и лечение на невродегенеративни заболявания, протичащи с деменция и допълнително на център за диагноза и лечение на Паркинсонова болест. От дълги години този център е реферирал център за заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, особено за комплексни, редки и наследствени случаи. През годините вследствие на натрупания опит и труд, както и значителен брой на пациенти с тези редки заболявания, реферирани към центъра са осъществени няколко дисертации в областта: 1. Когнитивни нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Мария Петрова, 2010 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Лонгитудинално проследяване на когнитивните нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Явор Желев, 2012 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. Клинико-генетични корелации при невродегенеративни заболявания, протичащи с паркинсонизъм (защитена дисертация за доктор по медицина от д-р Радка Павлова, 2013 г., ръководител: чл.-кор. проф. Лъчезар Трайков). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, протичащи с паркинсонизъм с и без когнитивен дефицит с подробно фенотипизиране на всеки един случай, което дава възможност за добър мониторинг на пациентите, както и изследователски анализ върху характеристиката на отделните заболявания. Дейността на центъра по отношение на диагноза и лечение на редки заболявания, протичащи с моторни и когнитивни нарушения, обхваща всички диагностични дейности съобразно новите диагностични критерии на тези заболявания, включително допълнителни изследвания, които са нужни за диференциална диагноза на атипични/ранни/наследствени случаи, включващи изследвания за биомаркери, невроизобразяващи и генетични фактори.

При 16 български пациенти е генетично верифициран Ювенилна Паркинсонова болест. Освен тези случаи, нашият център има кохорта от 52 български пациенти с Ювенилна Паркинсонова болест, при които не е установен генетичния дефект.

Публикации:

1. Pavlova R, Mehrabian S, Petrova M, Skelina S, Mihova K, Jordanova A, Mitev V, Traykov L. Cognitive, neuropsychiatric, and motor features associated with apolipoprotein E ε4 allele in a sample of Bulgarian patients with late-onset Parkinson's disease. Am J Alzheimers Dis Other Demen. 2014 Nov;29(7):614-9.
2. Petrova M, Raycheva M, Traykov L. Cognitive profile of the earliest stage of dementia in Parkinson's disease. Am J Alzheimers Dis Other Demen. 2012 Dec;27(8):614-9.
3. Petrova M, Raycheva M, Zhelev Y, Traykov L. Executive functions deficit in Parkinson's disease with amnestic mild cognitive impairment. Am J Alzheimers Dis Other Demen. 2010 Aug;25(5):455-60.
4. Kochev D, Petrova J, Petrova M, Krastev D, Traykov L. Possibility of combined assessment of biomarkers in early Parkinson's disease. International Journal of

Science and Research, 2014, 3, 10, 1332-1334;

5. Петрова М., Райчева М., Пенев Л., Григорова О., Желев Я., Трайков Л. Когнитивни различия между леко когнитивно нарушение и деменция при Паркинсонова болест. Българска Неврология, 2010, 4, 168-172.

6. Петрова М., Райчева М., Мехрабиан Ш., Желев Я., Ангов Г. Трайков Л. Връзки между депресията и когнитивните дефицити при пациенти с Паркинсонова болест и леко когнитивно нарушение. Българска Неврология, 2010, 10, 3, 122-125.
7. Петрова М., Трайков Л. Рискови фактори за развитие на когнитивни нарушения и деменция при Паркинсонова болест. Българска Неврология, 2010, 10, 3, 98-102.
8. Петрова М., Райчева М., Трайков Л. Връзки между предоминантния моторен подтип и когнитивни дефицити при пациенти с Паркинсонова болест с леко когнитивно нарушения. Българска Неврология, 2010, 4, 161-164.
9. Петрова М., Трайков Л. Особености в профила и диагностика на когнитивните нарушения при Паркинсонова болест, Неврология и Психиатрия, 2011, 1, 43.
10. Павлова Р, Мехрабиан Ш, Скелина С, Желев Я, Михова К, Кънева Р, Митев В, Йорданова А, Трайков Л. Характеристика на дегенеративния паркинсонов синдром в зависимост от Аполипопротеин Е генотипа. Неврология и психиатрия, 4, 30-33, 2014;
11. Петрова М., Григорова О, Желев Я., Павлова Р., Владимиров Б., Трайков Л. Влияние на Дуодопа върху моторните и немоторите усложнения при напреднала Паркинсонова болест. МЕДИКАРТ: Неврология и Психиатрия, 2014, 1, 24-29.
12. Кочев Д., Петрова Ю., Петрова М., Трайков Л. Оценка на ехогенността на субстанция нигра при пациенти с ранна Паркинсонова болест. Медицински Преглед, 2014, 50, 5, 45-47.
13. R. Pavlova, K. Mihova, S. Mehrabian, M. Petrova, S. Skelina, R. Kaneva, V. Mitev, L. Traykov. Novel LRRK2 6165C>G mutation in a patient with Parkinson's disease-dementia: a case report. In: JOINT CONGRESS OF EUROPEAN NEUROLOGY Istanbul, Turkey, May 31-June 3, 2014.
14. Pavlova R., K. Mihova, S. Mehrabian, M. Petrova, S. Skelina, R. Kaneva, A. Jordanova, V. Mitev, L. TraykovLRRK2 mutation c.4536+3A>G in a patient with multiple system atrophy: a case report. In: In: JOINT CONGRESS OF EUROPEAN NEUROLOGY Istanbul, Turkey, May 31-June 3, 2014.

ARTICLE

Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy

Suzanne Lesage,[1,2,3,4,25] Valérie Drouet,[1,2,3,4,25] Elisa Majounie,[5,25] Vincent Deramecourt,[6] Maxime Jacoupy,[1,2,3,4] Aude Nicolas,[1,2,3,4] Florence Cormier-Dequaire,[1,2,3,4,7] Sidi Mohamed Hassoun,[1,2,3,4] Claire Pujol,[1,2,3,4] Sorana Ciura,[1,2,3,4] Zoi Erpapazoglou,[1,2,3,4] Tatiana Usenko,[1,2,3,4] Claude-Alain Maurage,[6] Mourad Sahbatou,[8] Stefan Liebau,[9] Jinhui Ding,[5] Basar Bilgic,[10] Murat Emre,[10] Nihan Erginel-Unaltuna,[11] Gamze Guven,[11] François Tison,[12] Christine Tranchant,[13] Marie Vidailhet,[1,2,3,4,14] Jean-Christophe Corvol,[1,2,3,4,7] Paul Krack,[15] Anne-Louise Leutenegger,[16,17] Michael A. Nalls,[5] Dena G. Hernandez,[5] Peter Heutink,[18] J. Raphael Gibbs,[5] John Hardy,[19] Nicholas W. Wood,[19] Thomas Gasser,[18] Alexandra Durr,[1,2,3,4,20] Jean-François Deleuze,[21] Meriem Tazir,[22] Alain Destée,[23] Ebba Lohmann,[10,24] Edor Kabashi,[1,2,3,4] Andrew Singleton,[5] Olga Corti,[1,2,3,4,*] Alexis Brice,[1,2,3,4,20,*] French Parkinson's Disease Genetics Study (PDG), and the International Parkinson's Disease Genomics Consortium (IPDGC)

Autosomal-recessive early-onset parkinsonism is clinically and genetically heterogeneous. The genetic causes of approximately 50% of autosomal-recessive early-onset forms of Parkinson disease (PD) remain to be elucidated. Homozygozity mapping and exome sequencing in 62 isolated individuals with early-onset parkinsonism and confirmed consanguinity followed by data mining in the exomes of 1,348 PD-affected individuals identified, in three isolated subjects, homozygous or compound heterozygous truncating mutations in *vacuolar protein sorting 13C* (*VPS13C*). *VPS13C* mutations are associated with a distinct form of early-onset parkinsonism characterized by rapid and severe disease progression and early cognitive decline; the pathological features were striking and reminiscent of diffuse Lewy body disease. In cell models, VPS13C partly localized to the outer membrane of mitochondria. Silencing of *VPS13C* was associated with lower mitochondrial membrane potential, mitochondrial fragmentation, increased respiration rates, exacerbated PINK1/Parkin-dependent mitophagy, and transcriptional upregulation of *PARK2* in response to mitochondrial damage. This work suggests that loss of function of VPS13C is a cause of autosomal-recessive early-onset parkinsonism with a distinctive phenotype of rapid and severe progression.

Introduction

Parkinson disease (PD [MIM: 168600]) is a motor syndrome with variable combinations of akinesia, rigidity, and rest tremor responding to levodopa. It is caused by degeneration of the dopaminergic neurons in the substantia nigra pars compacta and is associated with Lewy bodies, intraneuronal inclusions enriched in α-synuclein. In recent years, our understanding of the pathophysiological mechanisms underlying molecular defects in familial forms of PD has greatly advanced. Three genes have been conclusively associated with autosomal-dominant (AD) forms of PD (*SNCA* [MIM: 163890], *LRRK2* [MIM: 609007], and *VPS35* [MIM: 601501]) and eight genes (*PARK2* [MIM: 602544], *PINK1* [MIM: 608309], *DJ-1* [MIM: 602533], *ATP13A2* [MIM: 610513], *FBXO7* [MIM: 605648], *PLA2G6* [MIM: 603604], *SYNJ1* [MIM: 604297], and *DNAJC6* [MIM: 608375]) with early-onset (EO) autosomal-recessive (AR) forms.[1] EO AR parkinsonism is clinically and genetically heterogeneous: mutations in *PARK2*, *PINK1*, and *DJ-1* cause phenotypes similar to idiopathic PD with good and prolonged response to dopaminergic

[1]Sorbonne Universités, UPMC Université Paris 6 UMR S 1127, 75013 Paris, France; [2]Inserm U 1127, 75013 Paris, France; [3]CNRS UMR 7225, 75013 Paris, France; [4]Institut du Cerveau et de la Moelle épinière, ICM, 75013 Paris, France; [5]Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD 20892, USA; [6]Department of Histology and Pathology, University of Lille Nord de France, Lille University Hospital, 59000 Lille, France; [7]Centre d'Investigation Clinique Pitié Neurosciences CIC-1422, 75013 Paris, France; [8]Fondation Jean Dausset-CEPH, 75010 Paris, France; [9]Institute of Neuroanatomy, Eberhard Karls University Tübingen, 72074 Tübingen, Germany; [10]Behavioural Neurology and Movement Disorders Unit, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, 34390 Istanbul, Turkey; [11]Department of Genetics, Institute for Experimental Medicine, Istanbul University, 34390 Istanbul, Turkey; [12]Institut des Maladies Neurodégénératives, Université de Bordeaux et CHU de Bordeaux, 33000 Bordeaux, France; [13]Pôle Tête-Cou-CETD, Service de Neurologie, Hôpitaux Universitaires, 67000 Strasbourg, France; [14]Pôle des Maladies du Système Nerveux, Fédération de Neurologie, Hôpital de la Salpêtrière, 75013 Paris, France; [15]Neurology Department, CHU de Grenoble, Joseph Fourier University, and INSERM U836, 38000 Grenoble, France; [16]Inserm U946, 75010 Paris, France; [17]Université Paris Diderot, Institut Universitaire d'Hématologie, UMR946, 75010 Paris, France; [18]Hertie Institute for Clinical Brain Research, University of Tübingen and DZNE, German Center for Neurodegenerative Diseases, 72074 Tübingen, Germany; [19]Department of Molecular Neuroscience, UCL Institute of Neurology, London WC1N 3BG, UK; [20]Department of Genetics and Cytogenetics, AP-HP, Hôpital de la Salpêtrière, 75013 Paris, France; [21]Commissariat à l'Energie Atomique, Institut Génomique, Centre National de Génotypage, 91000 Evry, France; [22]Service de Neurologie CHU Mustapha, 16000 Alger, Algérie; [23]Movement Disorders Unit, Lille University, Inserm U837, Lille University Hospital, 59000 Lille, France; [24]Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, and DZNE, German Center for Neurodegenerative Diseases, 72076 Tübingen, Germany
[25]These authors contributed equally to this work
*Correspondence: olga.corti@upmc.fr (O.C.), alexis.brice@upmc.fr (A.B.)
http://dx.doi.org/10.1016/j.ajhg.2016.01.014.

therapy. Other EO AR PD-associated genes cause more severe disease, a poor response to levodopa, and additional clinical signs, such as dystonia and cognitive impairment.[1] Mutations in *PARK2* and *PINK1* are the most common cause of EO AR PD, accounting for ~50% and 4% of familial cases in Europe, respectively.[2,3] A significant proportion of cases remain genetically unexplained.

EO AR PD is linked to mitochondrial dysfunction. The mitochondrial kinase PINK1 and the E3 ubiquitin-protein ligase Parkin cooperate in mitochondrial quality control.[4] They promote the removal of dysfunctional mitochondria in a process termed mitophagy that might also involve FBXO7.[4,5] In addition, they play a role in a vesicular trafficking pathway targeting damaged mitochondrial components to the lysosome.[6] To identify additional PD-associated genes involved in AR EO parkinsonism, we performed homozygosity mapping and exome sequencing in consanguineous PD families and isolated individuals and used data mining in the exomes of 1,348 unrelated PD-affected individuals. Five truncating mutations in *vacuolar protein sorting 13C* (*VPS13C* [MIM: 608879]) were identified in three unrelated PD-affected isolated individuals. We provide evidence that depletion of VPS13C exacerbates mitochondrial vulnerability to stress.

Subjects and Methods

Participants

Gene Discovery Cohort

We selected nine PD-affected families (with two or more affected siblings) and 43 unrelated isolated individuals according to the following criteria: (1) diagnosed by neurologists according to the UK Parkinson's Disease Society Brain Bank (PDSBB) clinical diagnostic criteria[7] and onset ≤ 55 years in at least one affected family member; (2) with no mutations in known PD-associated genes; and (3) with confirmed consanguinity (inbreeding coefficient $F \neq 0$ computed with the FEstim program[8]). Sixteen families or isolated subjects were European, 16 North African, 19 Turkish, and 1 Lebanese. A total of 66 PD-affected individuals (23 family members and 43 isolated subjects) and 39 unaffected relatives were included for the genome-wide screen study. 62 affected and 10 unaffected individuals were subsequently selected for whole-exome sequencing.

Validation Cohort

Exome data were obtained from a series of 1,348 additional PD-affected individuals (99% unrelated, 99% of European ancestry, 60% males, mean age at onset 41.7 ± 11.0 years), including 249 French PD-affected probands (57% males, age at onset ≤ 40 years, 50 individuals with atypical forms of parkinsonism) recruited by the French network for the study of PD genetics (PDG) and 530 matched control subjects (95% of European ancestry, 65% males, mean age at examination: 45.1 ± 10.7 years) from the International Parkinson Disease Genomics Consortium (IPDGC).

100 Turkish control subjects without family history of PD (43% males; mean age at examination, 60.4 ± 13.9 years) served to check for the absence of the identified variant in family A, which originated from Turkey.

A flow diagram detailing the selection criteria of PD-affected individuals and control subjects and the different experimental steps of the study is provided (Figure S1).

Study Approval

Informed consent was obtained from all participants, and the genetic studies were approved by local ethics committees (INSERM, CCPPRB du Groupe Hospitalier Pitié-Salpêtrière, Paris, France).

Neuropathological Assessment

The autopsy of the affected individual II-1 in family B (Figure 1) was performed approximately 36 hr post mortem. Brain tissues were fixed for 6 weeks in 10% buffered formalin, extensively sampled, and processed as previously described.[9] Immunohistochemistry was performed by a Ventana Benchmark automate. We used hematoxylin-eosin staining for histopathology. For immunochemistry, the antibodies used were: anti-Tau (in-house AD2,[10] 1 ng/mL), anti-β-amyloid (4G8, 1:1,000, Sigma), anti-α-synuclein (LB509, 1:500, Abcam), anti-ubiquitin (1:1,000, Dako), anti-TDP-43 (1:500, Protein Tech), and anti-glial fibrillary acid protein (GFAP, 1:20,000, Dako). The degree of neuronal loss and the frequency of α-synuclein-immunoreactive and other inclusions were determined semiquantitatively by visual inspection, in comparison to brains of three age-matched controls (two males and one female) from the Lille Neurobank collection.

Molecular Studies

Whole-Genome Homozygosity Linkage Mapping

Genome-wide screens were performed on all available affected (n = 66) and unaffected (n = 39) individuals from the gene discovery cohort using the Illumina HumanCytoSNP-12 v2.1 DNA Analysis BeadChip microarrays that contain ~300,000 SNPs and ~1,300 markers of common copy-number variations (CNVs). Homozygosity tracks (>2 Mb) were visualized with the Homozygosity Detector module and CNV with the Illumina cnvPartition module. The inbreeding coefficients F were computed with the FEstim program.[8] Samples from individuals with confirmed consanguinity ($F \neq 0$) were subjected to exome sequencing.

Whole-Exome Sequencing

Exons from 62 affected (19 relatives and 43 isolated individuals) and 10 unaffected family members from the gene discovery cohort and all the 1,348 affected and 530 control individuals from the validation cohort were captured using different exome enrichment kits from fragmented genomic DNA and sequenced as indicated in Table S1. 10-fold mean sequencing depth was achieved in 96.4% and 88.8% of baited regions in PD-affected individuals and control subjects, respectively, and 30-fold mean sequencing depth was achieved across ~75% of targeted regions.

Human reference sequence UCSC hg19 was used for sequence alignment and variant calling with the Burrows-Wheeler Aligner[11] and the Genome Analysis Toolkit.[12] PCR duplicates were removed prior to variant calling using Picard software. Variants were annotated with ANNOVAR software[13] (exomes from validation cohort) or SnpEff and SnpSift programs (exomes from gene discovery cohort). Data were analyzed with Ingenuity Variant Analysis (IVA) TM software from Ingenuity System. Effects on mRNA splicing by putative splice variants (±5 base pairs around splice junctions according to IVA threshold) were analyzed with Splice Site Finder, MaxEntScan, NNsplice, Genesplicer, and Human Splicing Finder,[14–16] all included in Alamut v.3 software. The human VPS13C protein and its closest homologs were aligned with Alamut v.3 software, computed by Ensembl, and aligned with MUSCLE.

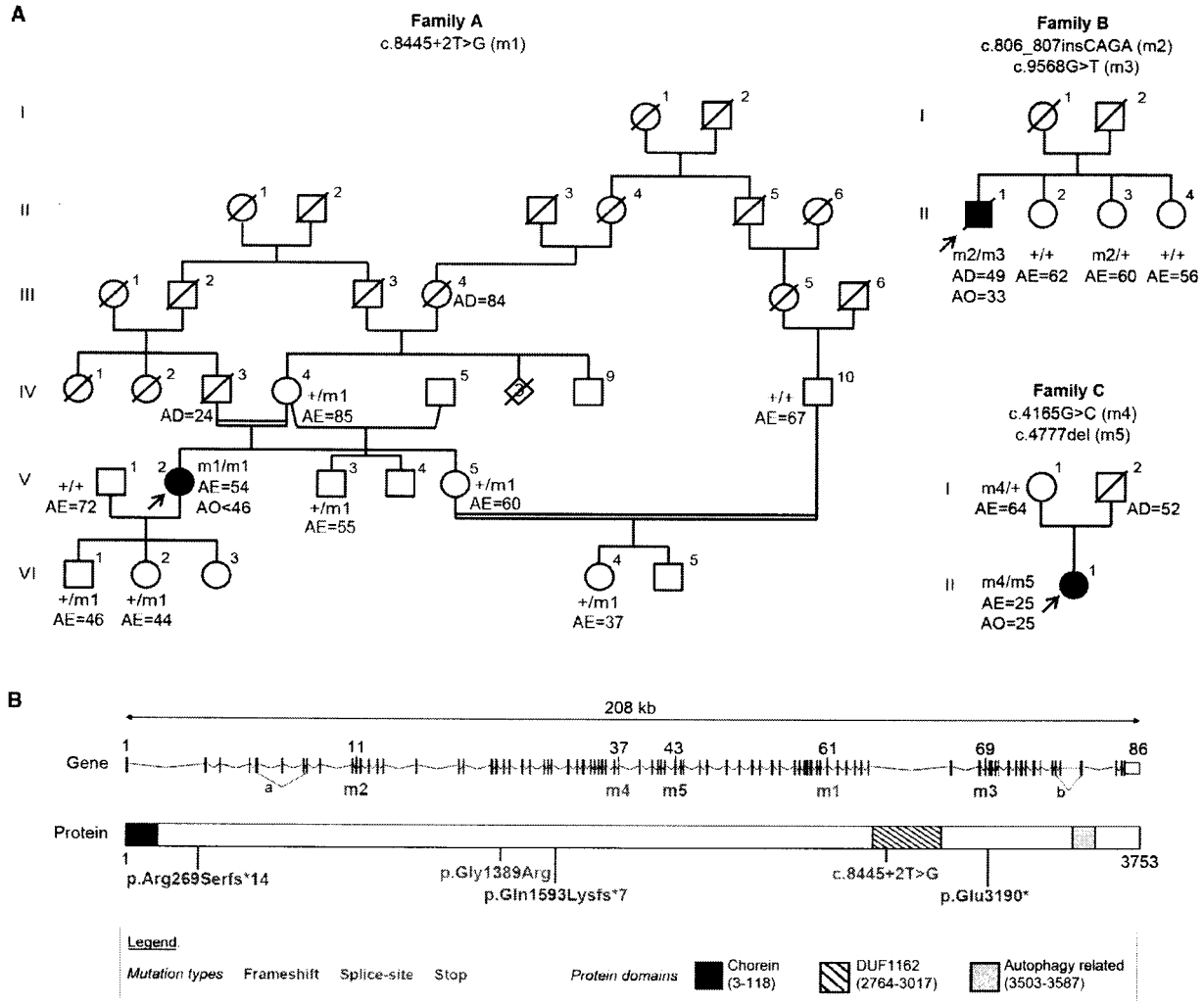


Figure 1. Identification of *VPS13C* Mutations

(A) Pedigrees of families with *VPS13C* mutations. Black symbols represent individuals with PD, open symbols, those unaffected. Arrows point to probands who underwent whole-exome sequencing. Abbreviations are as follows: AE, age at examination; AD, age at death; AO, age at onset.

(B) Schematic representation of *VPS13C* and its variations. *VPS13C* spans 208 kb and contains 86 exons encoding a 3,753-amino acid protein with a chorein domain at its N terminus, a DUF1162 domain of unknown function, and a putative autophagy-related domain. The five variations found in the three probands are indicated. Numbers above the gene identify the exons containing *VPS13C* variations. Alternative splicing a and b represent skipping of exons 6+7 and of exon 82, respectively. Transcripts 1A, GenBank: NM_017684.4: splicing a + b; 2A, GenBank: NM_020821.2: splicing b; 1B, GenBank: NM_018080.3: ends at exon 82; 2B, GenBank: NM_001018088.2: splicing a and ends at exon 82.

An in-house pipeline crossed the output data from IVA and Homozygosity Mapping to filter and identify the variants of interest (Figure S2). These variants were visualized with the Broad Institute Integrative Genomics Viewer (IGV) and verified by bidirectional Sanger sequencing using primers designed with Primer3 (Table S2) on an ABI 3730 automated sequencer (Life Technologies). Sanger sequencing confirmed the absence of mutations in origin-matched controls and determined the genetic status of unaffected relatives. Mutation nomenclature follows Human Genome Variation Society (HGVS) recommendations: the longest *VPS13C* transcript 2A cDNA nucleotides ("c.") are numbered from the adenine of the first ATG translation initiation codon as nucleotide +1 (reference sequence GenBank: NM_020821.2).

Splicing Defect Analysis by RT-PCR

Keratinocytes from affected individual V-2 in family A or peripheral blood lymphocytes from affected individual II-1 in family C (Figure 1) were used for splicing defects analyses. Hair follicles were plucked under sterile conditions and cultured in flasks coated with MG (1:10, Corning). Keratinocytes were grown on 20 mg/mL collagen IV (Sigma-Aldrich)-coated dishes containing EpiLife medium with the HKGS supplement (Life Technologies). Total RNA extraction was carried out with the RNeasy Kit (QIAGEN), according to the manufacture's manual. RNA (250 ng) was reverse transcribed into cDNA using iScript reverse transcription supermix (Bio-Rad). Primers are listed in Table S2. RT-PCR products were Sanger sequenced

directly or after sub-cloning into the pJET1.2/blunt vector (Thermo Scientific).

Studies in Mammalian Cells

Mammalian Expression Vectors, siRNAs, Cell Culture, and Transfection

COS-7 and HEK293T cells were grown in Dulbecco's Modified Eagle Medium + Glutamax (Life Technologies) supplemented with 10% goat serum (Life Technologies) and 1% penicillin-streptomycin (Life Technologies). Cells were plated, at 80% confluence, on glass coverslips (Thermo Scientific) in 24-well cell plates for immunofluorescence, 6-well plates for qPCR, 10-cm Petri dishes for subcellular fractionation by differential centrifugation and for the mitochondrion isolation kit, or 75-cm^2 flasks for continuous sucrose gradient and Percoll gradient purification. Cells were co-transfected with siRNAs (15 to 30 nM) and expression vectors using Lipofectamine 2000 (Life Technologies), in an antibiotic-free medium, according to the manufacturer's instructions. The siRNA used were: Hs_VPS13C_5 and Hs_VPS13C_6 (si*VPS13C*, QIAGEN); PINK1 stealth siRNAs (si*PINK1*, Invitrogen); and AllStars negative control siRNA (siControl, QIAGEN). Their efficacy was controlled by quantitative real-time RT-PCR (Figure 5F). The expression vectors were: pcDNA3-HA-PARK2, pcDNA3-HA-PINK1, pcDNA3-HA,[17] and pEGFP-C1 (Life Technologies). Where indicated, the cells were incubated with 10 μM CCCP (Sigma).

Subcellular Fractionation, Trypsin Digestion Assay, and Western Blot Analyses

For sucrose density gradient, confluent HEK293T cells from five 75-cm^2 flasks were harvested and disrupted with a Dounce homogenizer (80 manual strokes) in 10 mM Tris-HCl buffer (pH 7.6) containing 10% w/v sucrose, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM dithiothreitol (DTT), supplemented with protease and phosphatase inhibitors (0.2 mM sodium orthovanadate, 4 mg/mL sodium fluoride, 5.4 mg/mL β-glycerophosphate, and Complete cocktail 1X – 11836145001, Roche). After three centrifugations at 600 × *g* for 5 min to remove cell debris, the cell lysate was layered on a 20%–60% linear sucrose gradient in Tris-HCl (pH 7.6) containing 10 mM EDTA, as previously described.[18] After 18 hr of centrifugation at 100,000 × *g*, successive 0.8 mL fractions were collected. Proteins were precipitated on ice with 10% trichloroacetic acid, pelleted by centrifugation at 13,000 × *g* for 45 min, and resuspended in 100 μL of loading buffer (Tris 60 mM [pH 6.8], SDS 4%, β-mercaptoethanol 5%, glycerol, and bromophenol blue).

Total protein fractions were obtained from cells lysed in 210 mM mannitol, 70 mM sucrose, 5 mM Tris (pH 7.4), 0.2 mM EGTA, 0.1 mM EDTA, 0.5 mM DTT, 0.1% BSA, and protease and phosphatase inhibitors after centrifugation at 600 × *g* for 5 min at room temperature. Mitochondrion-enriched fractions were obtained by differential centrifugation (HEK293T) or magnetic isolation (COS-7) and digested with trypsin (Sigma), as previously described.[17,19]

For isolation of pure mitochondria, cells were lysed in 250 mM mannitol, 5 mM HEPES (pH 7.4), 5 mM EGTA with protease, and phosphatase inhibitors and the crude mitochondrial fraction was layered on top of a 30% Percoll gradient, as previously described.[20] Protein concentrations were determined with Bio-Rad protein assays (Bio-Rad, 500-0006), based on the Bradford method. Samples were boiled in protein sample buffer, resolved by SDS-PAGE, transferred onto a nitrocellulose membrane (Protran, Whatman), and analyzed by Western blotting with selected primary and secondary antibodies (Table S3). Membranes were incubated with enhanced chemiluminescence substrate (Pierce); chemiluminescent and fluorescent signals were revealed on film (ECL, Amersham Hyperfilm) or captured with Odyssey Imaging (Li-COR) systems and quantified with ImageJ software (NIH). Total or cytoplasmic fractions were normalized to α-tubulin, mitochondrial fractions to PMPCB. Three to six independent fractionation experiments were quantified.

Analysis of Mitochondrial Respiration

Cellular oxygen consumption was measured via high-resolution respirometry (OROBOROS Oxygraph-2k) in a temperature-regulated chamber at 37°C. Oxygen consumption was measured in intact COS-7 cells at a density of 2.5 × 10^6 cells in 2 mL of respiration assay medium (1× DMEM, GlutaMAX, GIBCO) containing 4.5 g/L D-glucose and 4 mM L-glutamine, by sequential additions of 1 μg/mL oligomycin, 2.5 μM CCCP, and 5 μM rotenone/10 μM antimycin A. We determined the following mitochondrial parameters: basal oxygen consumption (= basal cellular respiration – non-mitochondrial respiration), proton leak (= oligomycin-inhibited respiration – non-mitochondrial respiration), maximal respiratory capacity (= maximal uncoupled respiration – non-mitochondrial respiration), reserve respiratory capacity (= maximal uncoupled respiration – basal respiration), and non-mitochondrial respiration (rotenone/antimycin A-inhibited respiration). Cells were then lysed to quantify the protein content using the Bradford reagent, which was used to normalize the oxygen consumption data. The results were expressed in pmol of O_2/s/mg of total protein.

Immunostaining, $\Delta\Psi$mt, Respiration, Mitochondrial Morphology, and Parkin-Dependent Mitophagy

Immunocytochemical stainings were performed as described previously with the antibodies and dilution conditions indicated in Table S3. Changes in $\Delta\Psi$mt were evaluated with the potentiometric dye tetramethylrhodamine methyl ester (TMRM) as described.[19] Mitochondrial morphology was analyzed on COS-7 cells immunostained for the Beta subunit of the mitochondrial processing peptidase (PMPCB) using an image-processing algorithm and two descriptive parameters to assess mitochondrial length and branching: aspect ratio, calculated as the ratio between major and minor axes of each mitochondrial object, representing its length; and form factor, calculated as perimeter^2/(4π × area), representing a combined evaluation of the length and degree of branching of the mitochondrial network.[21,22] For Parkin-dependent mitophagy, cells were immunostained for PMPCB or the outer mitochondrial membrane protein TOMM20 and quantified as described.[17] Images were acquired with an Olympus FV-1000 confocal microscope (×60 oil immersion objective, NA 1.35) and analyzed with ImageJ analysis software (NIH).

Quantitative Real-Time RT-PCR

To demonstrate the efficiency of the siRNA-mediated silencing of endogenous *VPS13C* in HEK293T cells, total RNA was isolated from cells transfected with control or *VPS13C* or *PINK1* siRNA using the RNeasy plus Mini Kit (QIAGEN) and QIAshredder (QIAGEN). RNA from each sample (500 ng) was reverse transcribed into cDNA using iScript reverse transcription supermix (Bio-Rad). Real-time PCR was performed with the LightCycler 480 System (Roche Applied Science) and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). Results were analyzed with LightCycler 480 sw 1.5 quantification Software (Roche Applied Science). *Beta-actin* (ACTB) was used as the reference gene for normalization. Primers are listed in Table S2.

Statistical Analysis

Statistical significance was established at $p < 0.05$ and determined with an unpaired t test in Figures 4A (Aspect Ratio) and S6A; matched t test in Figures 4C, 5B, and 5D; Mann-Whitney-Wilcoxon test in Figure 4A (Form Factor); one-way ANOVA in Figures 6 and S5; or two-way ANOVA in Figures 4B, 5E, and 5F.

Results

Truncating Mutations in *VPS13C* Cause AR Parkinsonism

Genome-wide screens in an initial series of 66 affected and 39 unaffected subjects using DNA microarrays identified a mean of 16.3 regions of homozygosity $\geq$ 2 Mb on the 22 autosomes of each consanguineous individual (gene discovery cohort). No rare deleterious large genomic rearrangements were detected. Exomes were subsequently sequenced in the 62 affected individuals with confirmed consanguinity (inbreeding coefficient $F \neq 0$) and 10 unaffected family members to identify homozygous variants which: (1) in priority, would disrupt the protein function (frameshifts, stop codons, or splicing variants); (2) were rare (minor allele frequency [MAF] < 1%) in dbSNP137, the National Heart Lung and Blood Institute (NHBLI), Exome Sequencing Project (ESP) database, and the 1000 Genomes Project; (3) were shared by affected siblings when available; (4) were heterozygous in parents and/or heterozygous or wild-type in unaffected siblings when available; (5) fell within homozygous intervals; and (6) were absent in the homozygous state from DNA of 530 controls. We identified rare or undescribed homozygous truncating variants within 32 genes, each found in a single affected individual with consanguinity that fulfilled all these prioritization criteria (Table S4). We screened these candidate genes for additional homozygous or compound heterozygous mutations in a validation cohort. *VPS13C* (also known as KIAA1421; GenBank: NM_020821.2) on chr15q22 was mutated in a consanguineous Turkish PD-affected individual (V-2 in family A) from the gene discovery cohort and in two additional French PD-affected isolated individuals (II.1 in family B and II.1 in family C) from the validation cohort (Figure 1A). In addition, we identified a total of 80 rare (MAF < 1% in public databases) single heterozygous mostly nonsynonymous variants in *VPS13C* from the validation cohort, including 14 present in at least one of the 530 European control subjects (Table S5). No additional homozygous or compound heterozygous variants were found in the 31 other candidate genes.

The affected individual V-2 in family A harbored a homozygous splice-site mutation c.8445+2T>G, intron 61 in *VPS13C* confirmed by Sanger sequencing; eight unaffected relatives, including the mother, had heterozygous c.8445+2T>G mutations or wild-type sequences (Figures 1A and S3A). Affected individuals in families B and C were compound heterozygotes (Figures 1A, S3B, and S3C): the affected individual II-1 in family B with c.[806_807insCAGA];[9568G>T], p.[Arg269Serfs*14];[Glu3190*] variants; the affected individual II-1 in family C with c.[4165G>C];[4777delC], p.[Gly1389Arg];[Gln1593Lysfs*7] variants. Direct sequencing of *VPS13C* in the three unaffected siblings (II-2, II-3, and II-4) in family B and the unaffected mother (I-1) in family C showed that they all carried heterozygous mutations or wild-type alleles (Figures 1A and S3), indicating that all variations were located on different alleles. The five *VPS13C* variants were absent from dbSNP137, 1000 Genomes Project, EVS (Table S6A), Exome Aggregation Consortium (ExAC) databases (Table S6B), and our European control exomes, except for the missense p.Gly1389Arg variant found on one control chromosome. In addition, the c.8445+2T>G mutation was absent from 200 Turkish control chromosomes. No disruptive bi-allelic variants were found in our 530 control subjects; one disruptive homozygous variant (rs199602573) was found in the EVS database and ExAC populations (1/6,246 and 2/61,547, respectively), indicating that *VPS13C* homozygous disruptive variants are extremely rare in non-PD-affected populations (Tables S6 and S7).

The c.4165G>C and c.8445+2T>G mutations were predicted in silico to modify donor splice sites, one base upstream and two bases downstream, respectively, of splice junctions (Figure S4A). Reverse-transcription PCR analysis of potential splicing defects confirmed the predictions (Figure S4B). RNA from the homozygous individual of family A showed at least three shorter transcripts, lacking up to 231 nucleotides at the end of the exon 61. In the subject with the heterozygous variant, shorter transcripts were barely visible, probably due to a high instability of these aberrant RNAs. In family C, the longer transcript was found in the subject with the heterozygous variant, containing 14 additional nucleotides from intron 37.

VPS13C contains 86 exons spanning a 208-kb genomic region and has two main transcript variants, 1A (GenBank: NM_017684.4) and 2A (GenBank: NM_020821.2) (Figure 1B). Although the transcript 1A, lacking exons 6 and 7 and encoding a 3,710-amino acid protein, is expressed in most tissues, including brain and peripheral blood cells, the longest transcript 2A encodes a brain-specific 3,753-amino acid protein.[23] Two additional isoforms with uncharacterized expression pattern are reported in Ensembl (GenBank: NM_018080.3 and NM_001018088.2) and lack the last four exons. All the isoforms contain the splice site variants found in families A and C. VPS13C contains a chorein domain at its N terminus, a DUF1162 domain of unknown function, and a putative autophagy-related domain (Figure 1B). Except for the c.8445+2T>G variant, which is located in the DUF1162 domain, none of the variants were found in the predicted domains.

Clinical and Pathological Characteristics of Affected Individuals Harboring *VPS13C* Mutations

The three affected individuals harboring *VPS13C* mutations had early disease onset (25 to <46 years) and typical parkinsonism (akineto-rigid syndrome, rest tremor, good

Table 1. Clinical Characteristics of Affected Individuals Harboring *VPS13C* Mutations

	V-2 from Family A	II-1 from Family B	II-1 from Family C
Origin	Turkish	French	French
Consanguinity	yes	no	no
Gender	female	male	female
Age at onset (years)	<46	33	25
Symptoms at onset	depression; asymetric akineto rigid syndrome; no dystonia	asymetric akineto rigid syndrome and rest tremor; limb dystonia	asymetric akineto rigid syndrome; limb dystonia
Response to levodopa	yes, partial	yes, at early stage	yes, at early stage (evaluated at 75% initially)
Complications with treatment	no motor fluctuation nor dyskinesia	fluctuation and dyskinesia, ICD, somnolence	no motor fluctuation nor dyskinesia
Evolution	severe with early cognitive decline with spatial disorientation (MMSE 21), slurred speech and hallucinations at the age of 51; axial symptoms (postural instability, FOG, and falls) and dysautonomia with urinary incontinence at the age of 54; bedridden, unable to speak, apathetic, confused, cachexic with dysphagia at the age of 58	severe with early cognitive decline (MMSE 18 at the age of 40); axial symptoms (FOG at the age of 35, falls at the age of 39); dysautonomia (at the age of 35); bedridden at the age of 43, death at the age of 49 of aspiration pneumonia	severe with early cognitive decline, slurred speech before the age of 39; severe axial symptoms at early stage; bedridden at the age of 31; gastrostomia at the age of 37. Subject's father died at 52 of a pancreatic cancer
Atypical symptoms associated	brisk tendon reflexes on the lower limbs but no pyramidal syndrome	motor neuron signs with pyramidal syndrome and limb atrophy at late stage	motor neuron signs with spastic tetraplegia
Cerebral MRI	asymmetric atrophy in frontal, parietal, and temporal areas at the expense of the left side	normal (performed at early stage)	normal (performed at early stage)

Abbreviations are as follows: ICD, impulse control disorder; MMSE, mini-mental state examination; FOG, freezing of gait.

levodopa response). Disease progression, however, was particularly severe, with early cognitive decline, loss of response to treatment, axial symptoms, and dysautonomia. Affected subjects were bedridden within 15 years of clinical onset. Pyramidal signs and motor deficits were observed in two affected individuals. Brain MRI was normal early in the disease, and then bilateral atrophy was observed in the frontal, parietal, and temporal lobes (Table 1). Post-mortem examination of the brain of the affected individual II-1 in family B, who died at age 49 of aspiration pneumonia, showed mild frontal atrophy, including the primary motor area (Figures 2A–2C). The pathology resembled diffuse Lewy body disease. Alpha-synuclein and ubiquitin positive-Lewy bodies were observed in the brainstem, limbic system, hippocampus, and all cortical associative areas, including the parieto-occipital region (Figures 2D–2F, Table S8). Tau-immunoreactive neurofibrillary tangles and neurites were seen in the brainstem, hippocampus, and primary motor cortex (Figure 2G, Table S8). There were no glial-, α-synuclein-, Aβ-, or TDP-43-immunoreactive inclusions.

Loss of *VPS13C* Function Affects Mitochondrial Morphology, Transmembrane Potential, and Respiration

To investigate the function of VPS13C, we explored its subcellular distribution in human HEK293T by sucrose gradient fractionation (Figure 3A). VPS13C was enriched in the low-density fractions 1 and 2 containing the early endosomal marker EEA1 and most of the cytosolic protein Parkin. VPS13C was also found in higher-density fractions containing membrane and soluble markers of the Golgi apparatus (GOLGA2), the ER (Calnexin, BiP), and mitochondria (TOMM70, PMPCB, PINK1). Here, it was most abundant in fractions 8–10, containing the greatest proportion of TOMM70 and PMPCB. The mitochondrial localization of VPS13C was confirmed in mitochondrion-enriched fractions and pure mitochondria from HEK293T and COS-7 cells (Figures 3B and 3C). Limited trypsin digestion of mitochondrion-enriched fractions caused concomitant loss of VPS13C and the outer mitochondrial membrane receptor TOMM70 under conditions preserving the outer mitochondrial membrane channel TOMM40 and mitochondrial matrix enzyme PMPCB, indicating that VPS13C is located on the mitochondrial surface (Figure 3C).

We then investigated the impact of loss of *VPS13C* function on mitochondrial morphology, transmembrane potential, and respiration, reported to be affected in models of *PINK1* or *PARK2* deficiency.[24,25] The siRNA-mediated silencing of *VPS13C* in COS-7 cells reduced *VPS13C* mRNA levels to no more than 25% of the control condition (Figure S6) and was associated with perinuclear redistribution of mitochondria and mitochondrial

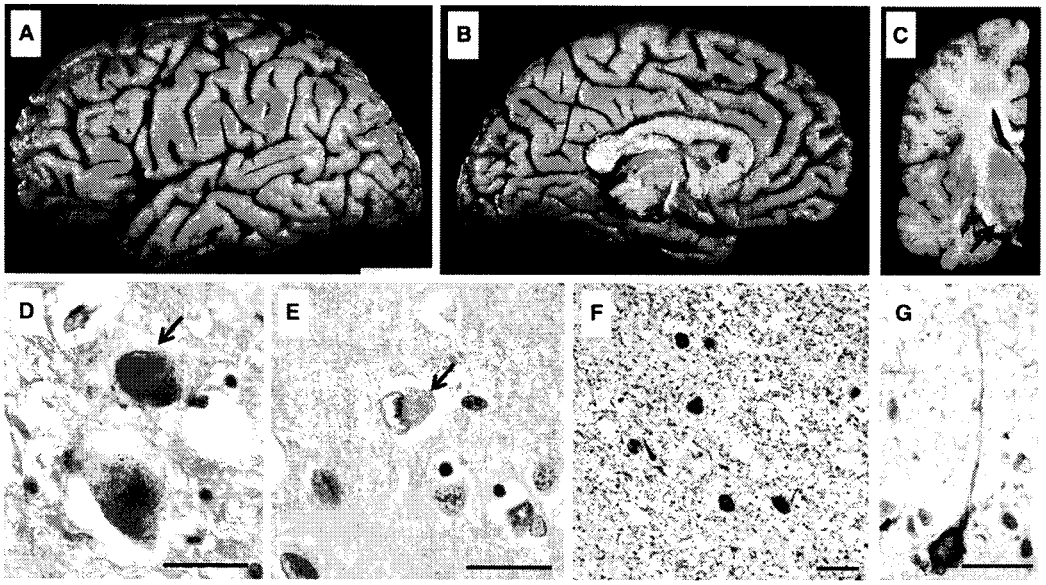


Figure 2. Neuropathology in the Proband of Family B with c.[806_807insCAGA];[9568G>T] *VPS13C* Mutations Shows Abundant α-Synucleinopathy

(A–C) Macroscopic appearance of the left hemisphere (fixed): lateral view (A); medial view (B); coronal section at the level of the cerebral peduncle (C).

(D and E) Lewy bodies in pigmented neurons in the substantia nigra (D, arrow, hematoxylin-eosin [HE] staining) and the parietal neocortex (E, arrowhead, HE staining).

(F) Representative image of α-synuclein immunoreactivity in the frontal cortex showing abundant Lewy bodies and neurites.

(G) Tau-immunoreactive neurofibrillary tangles in the primary motor cortex.

Scale bars for microscopic images represent 50 μm.

fragmentation, as confirmed by quantitative image analysis (Figure 4A). Evaluation of the mitochondrial transmembrane potential ($\Delta\Psi$mt) with the potentiometric dye tetramethylrhodamine methyl ester (TMRM) revealed a significant decrease in the mean fluorescence intensity of mitochondria in cells depleted of VPS13C (Figure 4B). The $\Delta\Psi$mt decrease was accompanied by an increase in maximal respiration rates and respiratory reserve, as assessed by high-resolution respirometry in intact cells (Figure 4C). Similar results were obtained in HEK293T cells (data not shown).

Loss of *VPS13C* Function Exacerbates PINK1/Parkin-Dependent Responses to Mitochondrial Depolarization

We further investigated the relationship between *VPS13C* and *PINK1* and *PARK2*, both at the transcript and protein levels, with respect to their well-characterized response to mitochondrial damage. PINK1 accumulates on mitochondria and recruits Parkin to initiate mitophagy in response to mitochondrial dysfunction.[4] Mitochondrial depolarization, triggered by the protonophore CCCP, partially redistributed VPS13C from mitochondria to the cytoplasm without significantly changing *VPS13C* transcript levels (Figures 5A, 5B, and 5F, left); under these conditions, PINK1 accumulated on mitochondria, as expected.[4] *VPS13C* silencing did not affect PINK1 levels under basal conditions, but it exacerbated CCCP-induced mitochondrial accumulation of PINK1 without impacting *PINK1* mRNA abundance (Figures 5C, 5D, and 5F, middle). Moreover, *VPS13C* silencing enhanced mitochondrial translocation of Parkin triggered by CCCP (Figures 5C and 5E). It also upregulated Parkin protein abundance in the cytosol without affecting *PARK2* transcript levels at 3 hr of CCCP treatment (Figures 5E and 5F, right). *PARK2* expression increases in response to mitochondrial damage caused by mitochondrial toxins, including CCCP.[26,27] Here, *PARK2* transcript levels tended to be higher at 48 hr of CCCP treatment (Figure 5F, right). This response was significantly enhanced after silencing of *VPS13C* or *PINK1*, suggesting greater mitochondrial damage. *PINK1* silencing was also associated with downregulation of *VPS13C* transcript levels under basal conditions (Figure 5F, left), an effect that was reversed by PINK1 overproduction (Figure S5), indicating the existence of multiple regulatory loops between VPS13C, PARK2, and PINK1. Consistent with the above described effects on PINK1 and Parkin, *VPS13C* silencing exacerbated PINK1/Parkin-mediated mitophagy triggered by CCCP in COS-7 cells, a model that we previously validated for the study of this process (Figures 6 and S6).[17,19]

Discussion

This study establishes *VPS13C* mutations as a monogenic cause of EO AR parkinsonism. Homozygous or compound

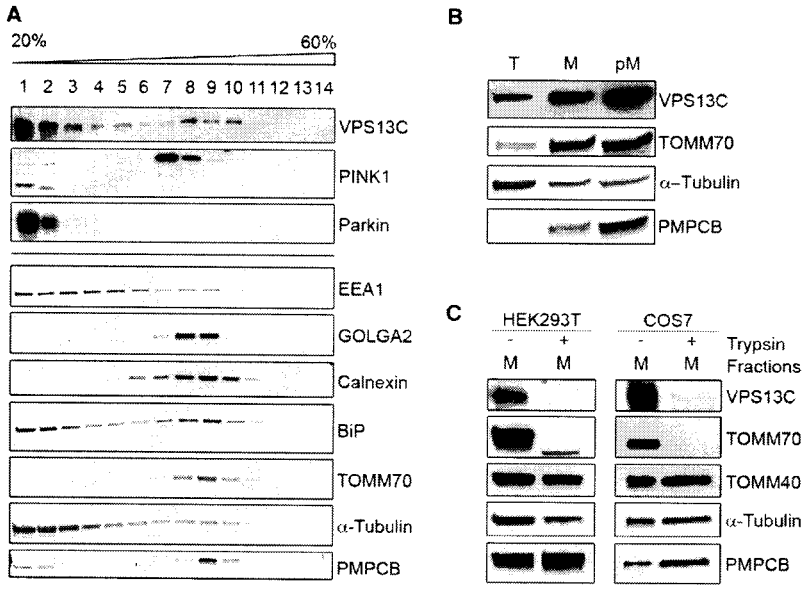


Figure 3. A Pool of VPS13C Is Located on the Outer Mitochondrial Membrane

(A) Sucrose gradient fractionation illustrating the subcellular distribution of endogenous VPS13C in HEK293T. Note the enrichment of the protein in fractions 1–3 and 8–10. Soluble endoplasmic reticulum (ER, BiP) and mitochondrial (PMPCB) markers in fractions 1 and 2 reflect organelle damage during fractionation.

(B) Western blot showing VPS13C immunoreactivity in mitochondria purified by Percoll gradient centrifugation from HEK293T cells (pM fraction). Note the enrichment in VPS13C and the mitochondrial markers TOMM70 and PMPCB in the pM fraction compared to the mitochondrion-enriched fraction (M). Abbreviation is as follows: T, total lysate.

(C) Limited trypsin treatment of mitochondrion-enriched fractions (M) from HEK293T or COS-7 cells caused loss of VPS13C and the mitochondrial surface marker TOMM70; the outer mitochondrial membrane channel TOMM40 and the matrix marker PMPCB are preserved.

heterozygous truncating mutations in three PD-affected individuals, absent from or present in the heterozygous state in available unaffected family members and in a very large number of control subjects, strongly support the pathogenicity of *VPS13C* in EO parkinsonism. We identified three affected individuals harboring *VPS13C* mutations and could not perform co-segregation analyses, due to the lack of additional affected relatives in the corresponding families. However, the affected individuals shared a specific, rare, and extremely distinctive phenotype consisting of EO parkinsonism with very rapid progression and dementia, which argues strongly for the pathogenicity of *VPS13C* mutations. The initial phenotype, EO parkinsonism and a good response to levodopa treatment, is similar to that of PD-affected individuals with *PARK2*, *PINK1*, or *DJ-1* mutations. However, the affected individuals rapidly became bedridden because of the worsening of motor dysfunction and loss of response to treatment. Dysautonomia and pyramidal signs were observed in two affected individuals, also distinguishing the phenotype from the classical, slowly progressive EO PD. The presence of numerous α-synuclein and ubiquitin-positive-Lewy bodies in the brainstem, limbic system, and many cortical areas was reminiscent of diffuse Lewy body disease, consistent with a motor phenotype associated with dementia. α-synuclein Lewy bodies are absent in most PD-affected individuals with *PARK2* mutations,[28] but were observed in the single autopsy case subject with *PINK1* mutations reported.[29] Tau-immunoreactive neurofibrillary tangles and neurites were also observed in case subjects with *PARK2* mutations.[28] These features define *VPS13C*-associated EO parkinsonism as a clinical, pathological, and genetic entity belonging to the group of synucleinopathies. In addition, our study also provides 31 potential candidate genes harboring disruptive homozygous mutations in a single PD-affected individual. However, genetic replication and functional validation are still needed to confirm their relevance to PD.

Alterations in other members of the *VPS13* family cause AR neurodegenerative disorders: *VPS13A* (MIM: 605978) (CHAC [MIM: 200150]) is mutated in chorea-acanthocytosis characterized by progressive neurodegeneration and red cell acanthocytosis,[30] and *VPS13B* (MIM: 607817) (COH1 [MIM: 216550]) is mutated in Cohen syndrome characterized by psychomotor retardation, microencephaly, and eye abnormalities.[31] *VPS13A*, *VPS13B*, and *VPS13C* are also mutated in gastric and colorectal cancers with unstable microsatellites.[32]

VPS13C belongs to a family of large VPS13 proteins (VPS13A–D) similar to yeast Vps13p.[23] Like yeast Vps, mammalian vacuolar sorting proteins are crucial for vesicular transport.[33] Initial studies linked yeast VPS13 orthologs to the delivery of proteins to the vacuole, the mammalian lysosome equivalent.[34] Mutations in *VPS35*, encoding a core component of the retromer complex regulating endosomal protein sorting, are implicated in AD late-onset PD.[35,36] VPS35 is also involved in protein trafficking from mitochondria to peroxisomes through mitochondria-derived vesicles.[37] PINK1 and Parkin play a role in this transport route, which delivers damaged mitochondrial cargo directly to lysosomes in response to mitochondrial stress.[6] However, the machinery regulating cargo selection and sorting into vesicles remains to be identified. VPS13C might be involved in this process. Such a mechanism would be consistent with its mitochondrial localization and the observed relocation to the cytosol in response to mitochondrial damage.

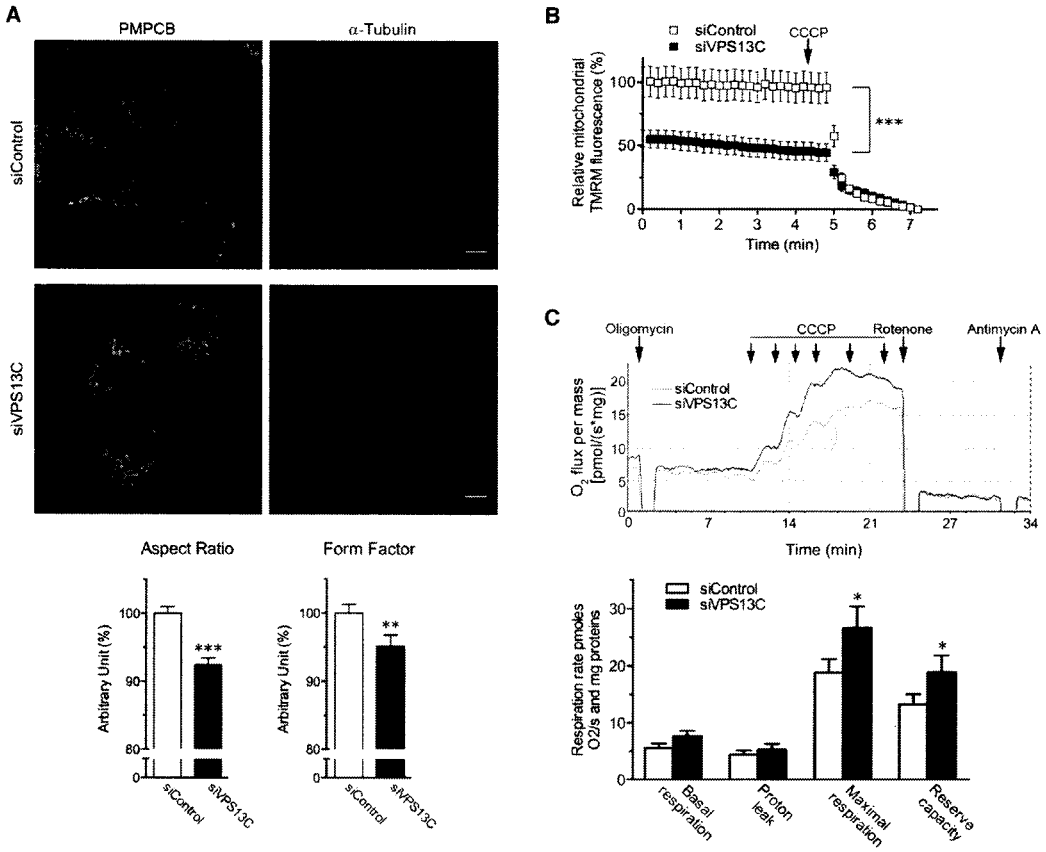


Figure 4. *VPS13C* Silencing Impacts Mitochondrial Morphology, Transmembrane Potential, and Respiration
(A) Representative immunofluorescence staining illustrating mitochondrial perinuclear redistribution and fragmentation in COS-7 cells silenced for *VPS13C* (si*VPS13C*, 30 nM) compared to cells treated with control siRNA (siControl, 30 nM): green, mitochondrial matrix marker PMPCB; red, α-Tubulin. *VPS13C* silencing reduced *VPS13C* mRNA levels to no more than 25% of the control condition (see Figure S6). Scale bars represent 10 μm. Quantification of aspect ratio and form factor (see Subjects and Methods, Koopman et al.[21], and Buhlman et al.[25]) shows reduced mitochondrial network complexity in si*VPS13C*-treated cells (means ± SEM; **$p < 0.01$; ***$p < 0.001$, of n = 88 or 86 cells scored per condition).
(B) Analysis of the relative TMRM fluorescence of mitochondria in COS-7 cells transfected as in (A), illustrating the decrease in ΔΨmt in cells depleted for VPS13C. n = 40 cells per condition from one experiment representative of three carried out. ***$p < 0.001$.
(C) Oxygen consumption rates in intact COS-7 cells transfected with siControl or si*VPS13C*. The top panel shows the oxygen flux corrected for instrumental background from one representative experiment. The graph in the bottom panel displays the respiration rates. Absence of *VPS13C* is associated with increased maximal respiration (= maximal uncoupled respiration under CCCP − non-mitochondrial respiration in the presence of the mitochondrial complex I and III inhibitors, rotenone, and antimycin A) and reserve capacity (= maximal uncoupled respiration − basal respiration before the addition of the complex V inhibitor oligomycin). Means ± SEM; *$p < 0.05$, of six independent experiments.

Several other observations in mammalian cells suggest that, like PINK1 and Parkin, VPS13C plays a role in mitochondrial maintenance. VPS13C depletion led to reduction of ΔΨmt and mitochondrial fragmentation in cell lines. Moreover, it enhanced maximal respiration rates, suggesting compensatory adaptation aimed at preserving ΔΨmt levels. In neuronal cells, which produce ATP mainly through mitochondrial oxidative phosphorylation and are unable to switch to glycolysis under acute mitochondrial stress, such changes might in the long term exacerbate generation of reactive oxygen species and trigger irreversible mitochondrial damage.[38,39] VPS13C depletion also upregulated PINK1/Parkin-dependent mitophagy, and, similarly to PINK1 depletion, it enhanced the previously reported transcriptional upregulation of Parkin in response to toxin-induced mitochondrial dysfunction. Overall, these data suggest that loss of *VPS13C* function increases mitochondrial vulnerability to stress and thereby activates PINK1/Parkin-dependent mitochondrial quality control pathways. Based on the inverse relationship between VPS13C and PINK1 protein levels on the mitochondrial surface, we cannot exclude that VPS13C also acts as a negative regulator of PINK1.

Mitochondrial function is ensured by a series of interconnected finely orchestrated pathways, activated in response to different degrees of mitochondrial dysfunction.[40] Excessive mitophagy has been associated with α-synuclein-dependent neurodegeneration.[41] Further work is required

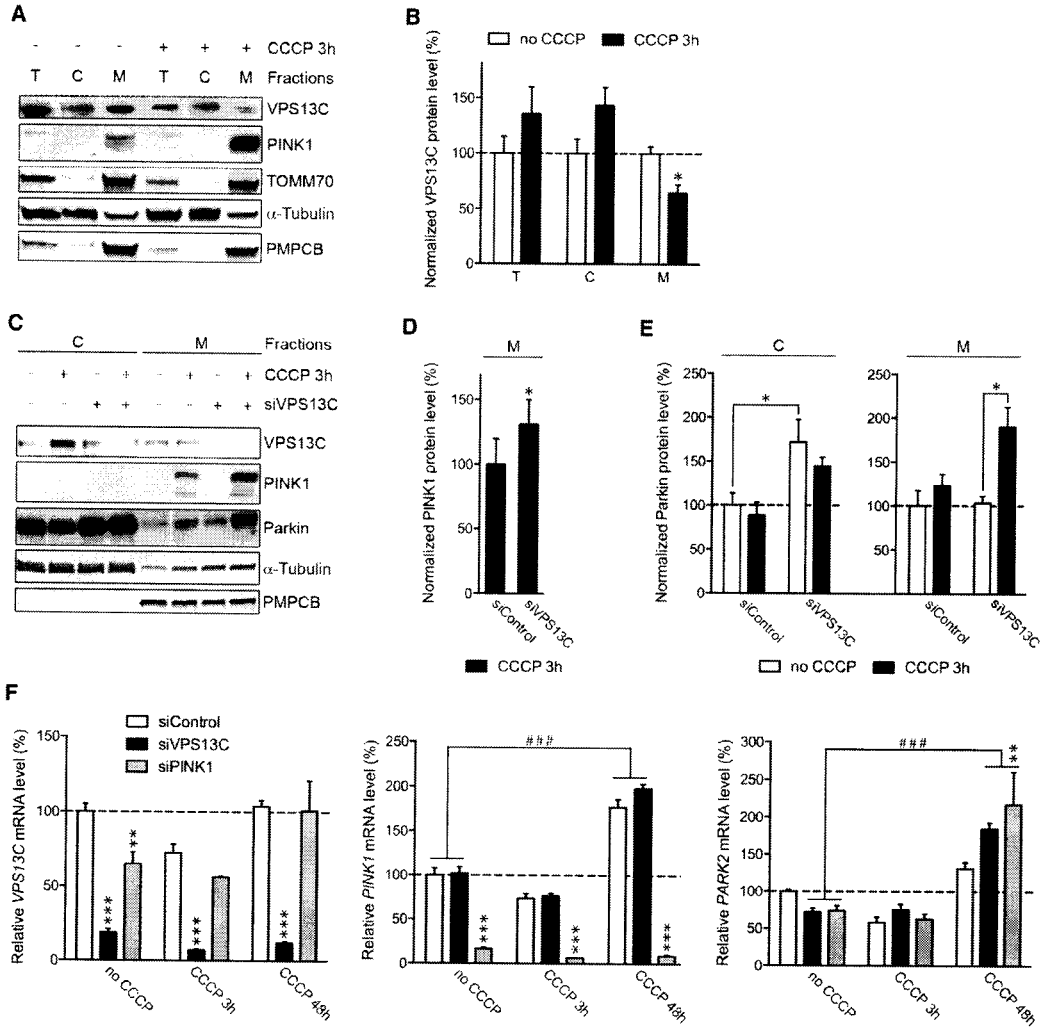


Figure 5. Loss of VPS13C Function Enhances Mitochondrial Accumulation of PINK1, Recruitment of Parkin, and *PARK2* Upregulation in Response to CCCP

(A and B) Western blot (A) and corresponding VPS13C protein levels (B) (normalized to α-Tubulin or PMPCB) in cytosolic (C), mitochondrion-enriched (M), and total (T) cell fractions from HEK293T cells treated or not with CCCP (10 μM, 3 hr). VPS13C levels decreased significantly in mitochondria after CCCP treatment, but tended to increase in cytosol (means ± SEM; *p < 0.05, of six independent fractionation experiments).

(C–E) Western blot (C) and corresponding normalized protein levels (D, E) in cytosolic and mitochondrion-enriched fractions from HEK293T transfected with 30 nM of control siRNA (siControl) or siRNA targeting *VPS13C* (si*VPS13C*).

(D) CCCP treatment resulted in accumulation of PINK1 (endogenous) in mitochondrion-enriched fractions (M) after treatment with siControl (−) and, more significantly, with si*VPS13C* (+).

(E) Accumulation of Parkin (endogenous) on depolarized mitochondria was also strongly enhanced in cells treated with si*VPS13C*. In addition, Parkin levels were upregulated in the cytosolic (C) fractions, particularly in untreated cells (means ± SEM; *p < 0.05 of four independent fractionation experiments).

(F) Quantitative real-time RT-PCR showing relative mRNA levels, normalized to α-actin (*ACTB*), in HEK293T cells treated with control siRNA (siControl), or siRNA targeting *VPS13C* (si*VPS13C*) or *PINK1* (si*PINK1*), under basal conditions or after CCCP treatment. Note the more than 30% decrease in *VPS13C* mRNA levels after *PINK1* silencing under basal conditions, but not after CCCP treatment (left). Note also that *VPS13C* and *PINK1* silencing enhance the upregulation of *PARK2* mRNA at 48 hr of CCCP treatment (right); means ± SEM of three to nine replicates per condition from two independent experiments (**p < 0.01; ***p < 0.001 compared to siControl within each condition of CCCP treatment; ###p < 0.001 between the indicated conditions of CCCP treatments).

to clarify the role of VPS13C in mitochondrial maintenance and dissect its possible relation to PINK1/Parkin-dependent pathways.

Enrichment of VPS13C in cell fractions containing the early endosomal marker EEA1 suggests broader roles for VPS13C in vesicular trafficking. A more general involvement in endosomal-lysosomal trafficking, possibly counteracting α-synuclein pathology as recently reported for VPS35,[42] might explain the diffuse α-synuclein pathology and rapid progression to dementia in individuals with *VPS13C*

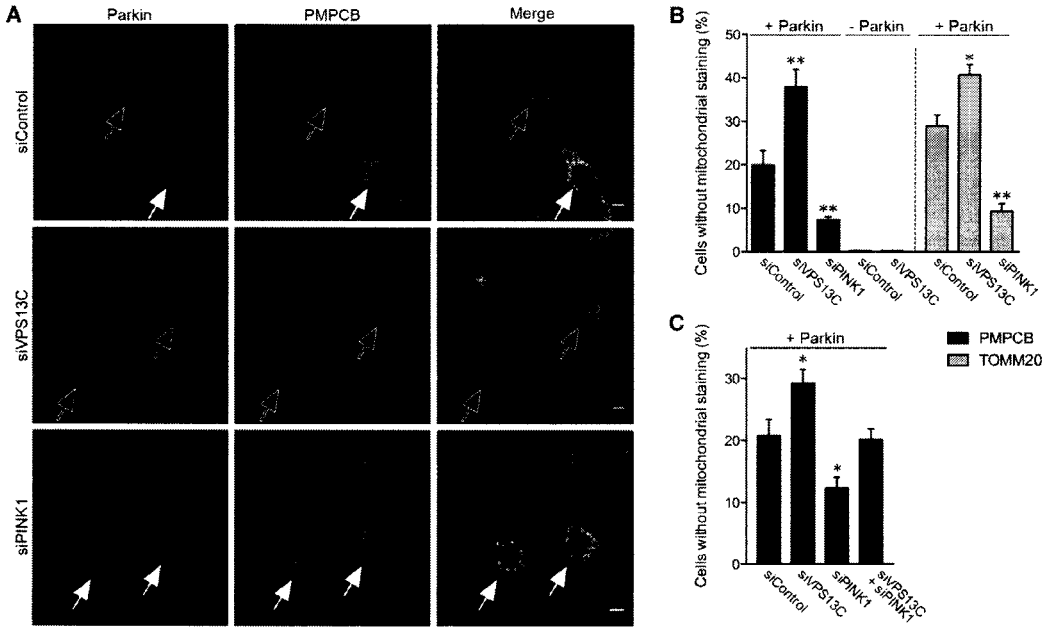


Figure 6. Loss of *VPS13C* Function Exacerbates PINK1/Parkin-Dependent Mitophagy

(A) Immunofluorescence staining of a representative experiment illustrating PINK1/Parkin-dependent mitophagy in COS-7 cells overproducing Parkin and silenced for *VPS13C* or *PINK1* (20 nM siRNA) after CCCP treatment (10 μM for 48 hr): red, Parkin; green, mitochondrial matrix marker PMPCB. Open arrows indicate loss of mitochondrial networks; white arrows show preserved networks. Scale bars represent 10 μm.

(B) Quantification of mitophagy in the conditions described in (A), expressed as the proportion of COS-7 cells without PMPCB (black bars) or TOMM20 (gray bars) staining; the si*VPS13C* treatment increased and si*PINK1* decreased the proportion. In the absence of exogenous Parkin (−Parkin; cells overproducing the control protein EGFP) or CCCP (not shown), all the cells harbored normal mitochondrial PMPCB staining, whether or not *VPS13C* was silenced (means ± SEM; *$p < 0.05$, **$p < 0.01$ of 3 independent experiments; 100 cells scored per condition).

(C) Proportion of COS-7 cells without PMPCB staining after transfection with half-doses (10 nM) of each siRNA and 48 hr of CCCP treatment. The mitophagy-promoting effect of *VPS13C* depletion was abolished by concomitant silencing of *PINK1* (means ± SEM; *$p < 0.05$ of 3 independent experiments; 100 cells scored per condition).

mutations. Such a mechanism would potentially represent a unifying link with cellular pathways involved in AD PD.

In summary, we describe truncating mutations in *VPS13C* associated with EO parkinsonism with rapid progression and widely distributed Lewy bodies. A meta-analysis of PD genome-wide association studies recently identified a susceptibility allele ~250 kb from *VPS13C* but not associated with either CpG methylation or mRNA expression,[43] suggesting that *VPS13C* can either cause a monogenic form of EO parkinsonism or confer genetic susceptibility to PD. Although we are confident that our work strongly implicates *VPS13C* mutations in PD, further genetic studies in other populations are needed to confirm their pathogenicity. The development of animal models in which *VPS13C* is stably inactivated will help dissect the mechanisms by which loss of *VPS13C* function affects the survival of dopaminergic neurons.

Accession Numbers

The accession numbers for the variations in *VPS13C* reported in this paper are ClinVar: SCV000262816, SCV000262817, SCV000262818, SCV000262819, and SCV000262820.

Supplemental Data

Supplemental Data include six figures and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.01.014.

Consortia

Members of The French Parkinson's Disease Genetics Study (PDG) are Suzanne Lesage, François Tison, Marie Vidailhet, Jean-Christophe Corvol, Yves Agid, Mathieu Anheim, Anne-Marie Bonnet, Michel Borg, Emmanuel Broussolle, Philippe Damier, Alain Destée, Alexandra Dürr, Franck Durif, Paul Krack, Stephan Klebe, Ebba Lohmann, Maria Martinez, Pierre Pollak, Olivier Rascol, Christine Tranchant, Marc Vérin, François Viallet, and Alexis Brice.

Members of The International Parkinson Disease Genomics Consortium (IPDGC) are Suzanne Lesage, Elisa Majounie, François Tison, Marie Vidailhet, Jean Christophe Corvol, Michael A. Nalls, Dena G. Hernandez, J. Raphael Gibbs, Alexandra Dürr, Sampath Arepalli, Roger A. Barker, Yoav Ben-Shlomo, Daniela Berg, Francesco Bettella, Kailash Bhatia, Rob M.A. de Bie, Alessandro Biffi, Bastiaan R. Bloem, Zoltan Bochdanovits, Michael Bonin, Jose M. Bras, Kathrin Brockmann, Janet Brooks, David J. Burn, Gavin Charlesworth, Honglei Chen, Patrick F. Chinnery, Sean Chong, Carl E. Clarke, Mark R. Cookson, Carl Counsell, Philippe Damier,

Jean-François Dartigues, Panos Deloukas, Günther Deuschl, David T. Dexter, Karin D. van Dijk, Allissa Dillman, Jing Dong, Frank Durif, Sarah Edkins, Valentina Escott-Price, Jonathan R. Evans, Thomas Foltynie, Jianjun Gao, Michelle Gardner, Alison Goate, Emma Gray, Rita Guerreiro, Clare Harris, Jacobus J. van Hilten, Albert Hofman, Albert Hollenbeck, Peter Holmans, Janice Holton, Michèle Hu, Xuemei Huang, Heiko Huber, Gavin Hudson, Sarah E. Hunt, Johanna Huttenlocher, Thomas Illig, Pálmi V. Jónsson, Laura L. Kilarski, Iris E. Jansen, Jean-Charles Lambert, Cordelia Langford, Andrew Lees, Peter Lichtner, Patricia Limousin, Grisel Lopez, Delia Lorenz, Steven Lubbe, Codrin Lungu, María Martinez, Walter Mätzler, Alisdair McNeill, Catriona Moorby, Matthew Moore, Karen E. Morrison, Ese Mudanohwo, Sean S. O'Sullivan, Michael J. Owen, Justin Pearson, Joel S. Perlmutter, Hjörvar Pétursson, Vincent Plagnol, Pierre Pollak, Bart Post, Simon Potter, Bernard Ravina, Tamas Revesz, Olaf Riess, Fernando Rivadeneira, Patrizia Rizzu, Mina Ryten, Mohamad Saad, Javier Simón-Sánchez, Stephen Sawcer, Anthony Schapira, Hans Scheffer, Claudia Schulte, Manu Sharma, Karen Shaw, Una-Marie Sheerin, Ira Shoulson, Joshua Shulman, Ellen Sidransky, Chris C.A. Spencer, Hreinn Stefánsson, Kári Stefánsson, Joanna D. Stockton, Amy Strange, Kevin Talbot, Carlie M. Tanner, Avazeh Tashakkori-Ghanbaria, Daniah Trabzuni, Bryan J. Traynor, André G. Uitterlinden, Daan Velseboer, Robert Walker, Bart van de Warrenburg, Mirdhu Wickremaratchi, Caroline H. Williams-Gray, Sophie Winder-Rhodes, Isabel Wurster, Nigel Williams, Huw R. Morris, Peter Heutink, John Hardy, Nicholas W. Wood, Thomas Gasser, Andrew B. Singleton, and Alexis Brice.

Acknowledgments

The authors are grateful to the families for their participation in this study. We thank Merle Ruberg for critical reading of the manuscript, the DNA and Cell Bank of ICM, the Plate-Forme d'Imagerie Cellulaire de la Pitié-Salpêtrière (PICPS), and Ebru Özer and Meltem Pak for sample preparation. We are grateful to the Lille brain bank for the gift of a brain ("Lille Neurobank," BB-0033-00030). This study was supported by the National Research Funding Agency (ANR-08-NEUR-004-01) in association with ERA-NET NEURON, the France-Parkinson Association, the Roger de Spoelberch Foundation (R12123DD), the French Academy of Sciences, the French program "Investissements d'avenir" (ANR-10-IAIHU-06), and the European Joint Programme - Neurodegenerative Disease Research (JPND-COURAGE-PD) project. This study was also supported by the Intramural Research Program of the National Institute on Aging and the National Institutes of Neurological Disorders and Stroke, NIH, Department of Health and Human Services (project Z01 AG000958 and by MRC Grant G1100643/1), by the European Social Fund, and by the Ministry of Science, Research, and the Arts, Baden-Württemberg. This work was also supported by the Department of Defense, including grant 10064005/11348001, the French Health Ministry (PHRC), France Parkinson Association, Lille University Hospital (A. Destée), the Atip/Avenir from the National Institute of Health and Medical Research (INSERM), the ANR in association with the ERA-NET E-rare program, the France Alzheimer Association, and a Career Integration Grant from Marie Curie Actions (E.K.). C.P. received a postdoctoral fellowship from the Cognacq-Jay Foundation. S.C. received postdoctoral fellowships from EMBO and AFM-Telethon.

Received: November 16, 2015
Accepted: January 20, 2016
Published: February 25, 2016

Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://browser.1000genomes.org
ANNOVAR, http://annovar.openbioinformatics.org/en/latest/
Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net/
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
Ensembl Genome Browser, http://www.ensembl.org/index.html
ExAC Browser, http://exac.broadinstitute.org/
IGV, http://www.broadinstitute.org/igv/
OMIM, http://www.omim.org/
GATK, https://www.broadinstitute.org/gatk/
Human Splicing Finder, http://www.umd.be/HSF3/HSF.html
Ingenuity Variant Analysis, http://www.ingenuity.com/products/variant-analysis
MUSCLE, http://www.ebi.ac.uk/Tools/msa/muscle/
NCBI Gene, http://www.ncbi.nlm.nih.gov/gene
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Picard, http://picard.sourceforge.net/
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
Primer3, http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi
SnpEff, http://snpeff.sourceforge.net/
SnpSift, http://snpeff.sourceforge.net/SnpSift.html
UCSC Genome Browser, http://genome.ucsc.edu

References

1. Bonifati, V. (2014). Genetics of Parkinson's disease–state of the art, 2013. Parkinsonism Relat. Disord. *20 (Suppl 1)*, S23–S28.
2. Lücking, C.B., Dürr, A., Bonifati, V., Vaughan, J., De Michele, G., Gasser, T., Harhangi, B.S., Meco, G., Denèfle, P., Wood, N.W., et al.; French Parkinson's Disease Genetics Study Group; European Consortium on Genetic Susceptibility in Parkinson's Disease (2000). Association between early-onset Parkinson's disease and mutations in the parkin gene. N. Engl. J. Med. *342*, 1560–1567.
3. Ibáñez, P., Lesage, S., Lohmann, E., Thobois, S., De Michele, G., Borg, M., Agid, Y., Dürr, A., and Brice, A.; French Parkinson's Disease Genetics Study Group (2006). Mutational analysis of the PINK1 gene in early-onset parkinsonism in Europe and North Africa. Brain *129*, 686–694.
4. Pickrell, A.M., and Youle, R.J. (2015). The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron *85*, 257–273.
5. Burchell, V.S., Nelson, D.E., Sanchez-Martinez, A., Delgado-Camprubi, M., Ivatt, R.M., Pogson, J.H., Randle, S.J., Wray, S., Lewis, P.A., Houlden, H., et al. (2013). The Parkinson's disease-linked proteins Fbxo7 and Parkin interact to mediate mitophagy. Nat. Neurosci. *16*, 1257–1265.
6. McLelland, G.-L., Soubannier, V., Chen, C.X., McBride, H.M., and Fon, E.A. (2014). Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. EMBO J. *33*, 282–295.
7. Hughes, A.J., Daniel, S.E., Kilford, L., and Lees, A.J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry *55*, 181–184.
8. Leutenegger, A.-L., Prum, B., Génin, E., Verny, C., Lemainque, A., Clerget-Darpoux, F., and Thompson, E.A. (2003).

Estimation of the inbreeding coefficient through use of genomic data. Am. J. Hum. Genet. *73*, 516–523.

9. Buée-Scherrer, V., Condamines, O., Mourton-Gilles, C., Jakes, R., Goedert, M., Pau, B., and Delacourte, A. (1996). AD2, a phosphorylation-dependent monoclonal antibody directed against tau proteins found in Alzheimer's disease. Brain Res. Mol. Brain Res. *39*, 79–88.
10. Deramecourt, V., Lebert, F., Maurage, C.-A., Fernandez-Gomez, F.-J., Dujardin, S., Colin, M., Sergeant, N., Buée-Scherrer, V., Clot, F., Ber, I.L., et al. (2012). Clinical, neuropathological, and biochemical characterization of the novel tau mutation P332S. J. Alzheimers Dis. *31*, 741–749.
11. Li, H., and Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics *26*, 589–595.
12. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. *20*, 1297–1303.
13. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. *38*, e164.
14. Crooks, G.E., Hon, G., Chandonia, J.-M., and Brenner, S.E. (2004). WebLogo: a sequence logo generator. Genome Res. *14*, 1188–1190.
15. Desmet, F.-O., Hamroun, D., Lalande, M., Collod-Béroud, G., Claustres, M., and Béroud, C. (2009). Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. *37*, e67.
16. Schneider, T.D., and Stephens, R.M. (1990). Sequence logos: a new way to display consensus sequences. Nucleic Acids Res. *18*, 6097–6100.
17. Bertolin, G., Ferrando-Miguel, R., Jacoupy, M., Traver, S., Grenier, K., Greene, A.W., Dauphin, A., Waharte, F., Bayot, A., Salamero, J., et al. (2013). The TOMM machinery is a molecular switch in PINK1 and PARK2/PARKIN-dependent mitochondrial clearance. Autophagy *9*, 1801–1817.
18. Erpapazoglou, Z., Froissard, M., Nondier, I., Lesuisse, E., Haguenauer-Tsapis, R., and Belgareh-Touzé, N. (2008). Substrate- and ubiquitin-dependent trafficking of the yeast siderophore transporter Sit1. Traffic *9*, 1372–1391.
19. Bertolin, G., Jacoupy, M., Traver, S., Ferrando-Miguel, R., Saint Georges, T., Grenier, K., Ardila-Osorio, H., Muriel, M.-P., Takahashi, H., Lees, A.J., et al. (2015). Parkin maintains mitochondrial levels of the protective Parkinson's disease-related enzyme 17-β hydroxysteroid dehydrogenase type 10. Cell Death Differ. *22*, 1563–1576.
20. Wieckowski, M.R., Giorgi, C., Lebiedzinska, M., Duszynski, J., and Pinton, P. (2009). Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. Nat. Protoc. *4*, 1582–1590.
21. Koopman, W.J.H., Verkaart, S., Visch, H.-J., van der Westhuizen, F.H., Murphy, M.P., van den Heuvel, L.W.P.J., Smeitink, J.A.M., and Willems, P.H.G.M. (2005). Inhibition of complex I of the electron transport chain causes O2-.-mediated mitochondrial outgrowth. Am. J. Physiol. Cell Physiol. *288*, C1440–C1450.
22. Buhlman, L., Damiano, M., Bertolin, G., Ferrando-Miguel, R., Lombès, A., Brice, A., and Corti, O. (2014). Functional interplay between Parkin and Drp1 in mitochondrial fission and clearance. Biochim. Biophys. Acta *1843*, 2012–2026.
23. Velayos-Baeza, A., Vettori, A., Copley, R.R., Dobson-Stone, C., and Monaco, A.P. (2004). Analysis of the human VPS13 gene family. Genomics *84*, 536–549.
24. Exner, N., Treske, B., Paquet, D., Holmström, K., Schiesling, C., Gispert, S., Carballo-Carbajal, I., Berg, D., Hoepken, H.-H., Gasser, T., et al. (2007). Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. J. Neurosci. *27*, 12413–12418.
25. Mortiboys, H., Thomas, K.J., Koopman, W.J.H., Klaffke, S., Abou-Sleiman, P., Olpin, S., Wood, N.W., Willems, P.H.G.M., Smeitink, J.A.M., Cookson, M.R., and Bandmann, O. (2008). Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. Ann. Neurol. *64*, 555–565.
26. Henn, I.H., Bouman, L., Schlehe, J.S., Schlierf, A., Schramm, J.E., Wegener, E., Nakaso, K., Culmsee, C., Berninger, B., Krappmann, D., et al. (2007). Parkin mediates neuroprotection through activation of IkappaB kinase/nuclear factor-kappaB signaling. J. Neurosci. *27*, 1868–1878.
27. Bouman, L., Schlierf, A., Lutz, A.K., Shan, J., Deinlein, A., Kast, J., Galehdar, Z., Palmisano, V., Patenge, N., Berg, D., et al. (2011). Parkin is transcriptionally regulated by ATF4: evidence for an interconnection between mitochondrial stress and ER stress. Cell Death Differ. *18*, 769–782.
28. Doherty, K.M., Silveira-Moriyama, L., Parkkinen, L., Healy, D.G., Farrell, M., Mencacci, N.E., Ahmed, Z., Brett, F.M., Hardy, J., Quinn, N., et al. (2013). Parkin disease: a clinicopathologic entity? JAMA Neurol. *70*, 571–579.
29. Samaranch, L., Lorenzo-Betancor, O., Arbelo, J.M., Ferrer, I., Lorenzo, E., Irigoyen, J., Pastor, M.A., Marrero, C., Isla, C., Herrera-Henriquez, J., and Pastor, P. (2010). PINK1-linked parkinsonism is associated with Lewy body pathology. Brain *133*, 1128–1142.
30. Rampoldi, L., Dobson-Stone, C., Rubio, J.P., Danek, A., Chalmers, R.M., Wood, N.W., Verellen, C., Ferrer, X., Malandrini, A., Fabrizi, G.M., et al. (2001). A conserved sorting-associated protein is mutant in chorea-acanthocytosis. Nat. Genet. *28*, 119–120.
31. Kolehmainen, J., Black, G.C.M., Saarinen, A., Chandler, K., Clayton-Smith, J., Träskelin, A.-L., Perveen, R., Kivitie-Kallio, S., Norio, R., Warburg, M., et al. (2003). Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. Am. J. Hum. Genet. *72*, 1359–1369.
32. An, C.H., Kim, Y.R., Kim, H.S., Kim, S.S., Yoo, N.J., and Lee, S.H. (2012). Frameshift mutations of vacuolar protein sorting genes in gastric and colorectal cancers with microsatellite instability. Hum. Pathol. *43*, 40–47.
33. Richardson, S.C.W., Winistorfer, S.C., Poupon, V., Luzio, J.P., and Piper, R.C. (2004). Mammalian late vacuole protein sorting orthologues participate in early endosomal fusion and interact with the cytoskeleton. Mol. Biol. Cell *15*, 1197–1210.
34. Bankaitis, V.A., Johnson, L.M., and Emr, S.D. (1986). Isolation of yeast mutants defective in protein targeting to the vacuole. Proc. Natl. Acad. Sci. USA *83*, 9075–9079.
35. Vilariño-Güell, C., Wider, C., Ross, O.A., Dachsel, J.C., Kachergus, J.M., Lincoln, S.J., Soto-Ortolaza, A.I., Cobb, S.A., Wilhoite, G.J., Bacon, J.A., et al. (2011). VPS35 mutations in Parkinson disease. Am. J. Hum. Genet. *89*, 162–167.
36. Zimprich, A., Benet-Pagès, A., Struhal, W., Graf, E., Eck, S.H., Offman, M.N., Haubenberger, D., Spielberger, S., Schulte, E.C., Lichtner, P., et al. (2011). A mutation in VPS35, encoding

a subunit of the retromer complex, causes late-onset Parkinson disease. Am. J. Hum. Genet. *89*, 168–175.

37. Braschi, E., Goyon, V., Zunino, R., Mohanty, A., Xu, L., and McBride, H.M. (2010). Vps35 mediates vesicle transport between the mitochondria and peroxisomes. Curr. Biol. *20*, 1310–1315.
38. Almeida, A., Almeida, J., Bolaños, J.P., and Moncada, S. (2001). Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. Proc. Natl. Acad. Sci. USA *98*, 15294–15299.
39. Almeida, A., Moncada, S., and Bolaños, J.P. (2004). Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. Nat. Cell Biol. *6*, 45–51.
40. Rugarli, E.I., and Langer, T. (2012). Mitochondrial quality control: a matter of life and death for neurons. EMBO J. *31*, 1336–1349.
41. Choubey, V., Safiulina, D., Vaarmann, A., Cagalinec, M., Wareski, P., Kuum, M., Zharkovsky, A., and Kaasik, A. (2011). Mutant A53T alpha-synuclein induces neuronal death by increasing mitochondrial autophagy. J. Biol. Chem. *286*, 10814–10824.
42. Dhungel, N., Eleuteri, S., Li, L.-B., Kramer, N.J., Chartron, J.W., Spencer, B., Kosberg, K., Fields, J.A., Stafa, K., Adame, A., et al. (2015). Parkinson's disease genes VPS35 and EIF4G1 interact genetically and converge on α-synuclein. Neuron *85*, 76–87.
43. Nalls, M.A., Pankratz, N., Lill, C.M., Do, C.B., Hernandez, D.G., Saad, M., DeStefano, A.L., Kara, E., Bras, J., Sharma, M., et al.; International Parkinson's Disease Genomics Consortium (IPDGC); Parkinson's Study Group (PSG) Parkinson's Research: The Organized GENetics Initiative (PROGENI); 23andMe; GenePD; NeuroGenetics Research Consortium (NGRC); Hussman Institute of Human Genomics (HIHG); Ashkenazi Jewish Dataset Investigator; Cohorts for Health and Aging Research in Genetic Epidemiology (CHARGE); North American Brain Expression Consortium (NABEC); United Kingdom Brain Expression Consortium (UKBEC); Greek Parkinson's Disease Consortium; Alzheimer Genetic Analysis Group (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat. Genet. *46*, 989–993.

Published in final edited form as:
Arch Neurol. 2008 April ; 65(4): 467–474. doi:10.1001/archneur.65.4.467.

Risk of Parkinson's Disease in Carriers of *Parkin* mutations: Estimation Using the Kin-Cohort Method

Yuanjia Wang, PhD[1], **Lorraine N. Clark, PhD**[2,3], **Elan D. Louis, MD MS**[2,4,5,7], **Helen Mejia-Santana, MS**[4], **Juliette Harris, PhD**[5], **Lucien J. Cote, MD**[4,5], **Cheryl Waters, MD FRCP**[5], **Howard Andrews, PhD**[4,7], **Blair Ford, MD FRCP**[5], **Steven Frucht, MD**[5], **Stanley Fahn, MD**[5], **Ruth Ottman, PhD**[4,5,7,9], **Daniel Rabinowitz, PhD**[8], and **Karen Marder, MD MPH**[2,4,5,6]

[1]Department of Biostatistics, Mailman School of Public Health, Columbia University New York, NY

[2]Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University New York, NY

[3]Department of Pathology, College of Physicians and Surgeons, Columbia University New York, NY

[4]Gertrude H. Sergievsky Center, Columbia University New York, NY

[5]Department Neurology, College of Physicians and Surgeons, Columbia University New York, NY

[6]Department Psychiatry, College of Physicians and Surgeons, Columbia University New York, NY

[7]Department of Epidemiology, Mailman School of Public Health, Columbia University New York, NY

[8]Department of Statistics, Columbia University New York, NY

[9]the Epidemiology of Brain Disorders Department, New York State Psychiatric Institute.

Abstract

Objective—To estimate the risk of Parkinson's disease in individuals with mutations in the *Parkin* gene.

Design—We assessed point mutations and exon deletions and duplications in the *Parkin* gene in 247 PD probands with age at onset ≤50 and 104 control probands enrolled in the Genetic Epidemiology of PD study. For each first-degree relative, a consensus diagnosis of PD was established. The probability that each relative carried a mutation was estimated from the proband's *Parkin* carrier status using Mendelian principles and the relationship of the relative to the proband .

Results—*Parkin* mutations were identified in 25 PD probands (10.1%), 72% of whom were heterozygotes. One *Parkin* homozygote reported 2 siblings with PD. The cumulative incidence of PD to age 65 in carrier relatives (age-specific penetrance) was estimated to be 7.0% (95% CI: 0.4-71.9%) compared to 1.7% (95% CI: 0.8-3.4%) in non-carrier relatives of cases (p=0.59) and 1.1% (95% CI: 0.3-3.4%). in relatives of controls (compared to non-carriers p=0.52).

Conclusions—The cumulative risk of PD to age 65 in a non-carrier relative of a case with AAO ≤50 is not significantly greater than the general population risk among controls. Age specific penetrance among *Parkin* carriers, in particular heterozygotes, deserves further study.

Correspondence to: Karen Marder MD MPH 630 W 168th St., Unit 16 New York New York 10032.

Keywords

Parkin; Mutations; Parkinson's disease; Kin-cohort study; Early onset

Introduction

Mutations in the *Parkin* gene (PARK2)[1, 2] are associated primarily with early-onset Parkinson's disease (EOPD), defined as age at onset (AAO) ranging from ≤45 to ≤55[3-7], but have also been described in PD cases with an AAO over 70 years [7-10]. In PD cases with AAO ≤ 45 with a mode of inheritance consistent with autosomal recessive transmission, the frequency of *Parkin* mutations may be as high as 49%[3] while in cases without a family history of PD the range is 15-18%.[4, 6] AAO is inversely correlated with the frequency of *Parkin* mutations in both familial[3] and sporadic[6] cases.

Several studies have compared the AAO of PD in heterozygous, compound heterozygous, and homozygous *Parkin* mutation carriers [3-10] and found that heterozygous cases, both familial and sporadic, have older AAO. Heterozygous *Parkin* mutation carriers are more frequently reported among sporadic than familial cases[7].

Information on the risk of PD in individuals who carry *Parkin* mutations in either the homozygous, compound heterozygous, or heterozygous state (or penetrance) is essential for genetic counseling. The penetrance of *Parkin* mutations has only been reported for isolated families[7]. Most of the previous study designs sampled PD cases based on family history of PD, which would bias penetrance estimates upwards[11, 12]. To obtain an unbiased estimate of risk, a population-based random sample would be desirable, but *Parkin* mutations are so rare in the population that such a sample would have to be extremely large to obtain sufficient precision in penetrance estimates.

To obtain unbiased estimates of the risk of PD in *Parkin* carriers despite the low population frequency of *Parkin* mutations [13], we used a kin-cohort study design[11, 12] applied to participants in the Genetic Epidemiology of Parkinson's Disease (GEPD) study[14]. The kin-cohort design is highly efficient for estimating penetrance, because the relatives' mutation status is not required for the analyses, thus reducing costs for genetic analysis[15].

Methods

Subjects

Details of the GEPD study have been previously described [14, 16, 17]. Cases were ascertained based on AAO of motor signs ≤50 (EOPD) or >50 (LOPD). In this study we included all 247 PD cases with AAO ≤50. All cases were recruited from the Center for Parkinson's Disease and Other Movement Disorders at Columbia University (CPD), and EOPD cases were oversampled[14]. One hundred and five controls were randomly selected from 412 controls in the GEPD study for complete sequencing of the *Parkin* gene.[18] The majority of the controls were recruited by random digit dialing, with frequency matching by age, gender, ethnicity and area code/exchange. An additional sample of 40 controls of Hispanic descent, 11 African American (AA) controls and 170 Caucasian controls participating in GEPD were used to examine Parkin variants that have not been previously described. All PD cases and control probands were seen in-person and underwent an identical evaluation[14] that included a medical history, Unified Parkinson's Disease Rating Scale (UPDRS)[19] and videotape assessment.

Diagnosis of PD in Relatives

Information on the family history of PD in first-degree relatives was obtained by administering a reliable, validated interview to each case, control, and first-degree relative. For relatives who were deceased or otherwise unavailable for interview, the history was obtained by interviewing the most knowledgeable informant[16]. An algorithm was created to generate a final diagnosis for PD in each first-degree relative based on the family history interview and the direct interview with the relative. For relatives diagnosed with PD, a level of certainty was assigned as definite, probable, possible, uncertain, and unlikely. For first-degree relatives who met criteria for uncertain, possible, probable or definite PD, we tried to obtain additional information in the form of an examination, medical records, or an independent interview by a neurologist. A best estimate diagnosis of PD was assigned for each relative [14]. Family history information including age at onset of PD was available for 1330 relatives of 224 PD cases and 638 relatives of 103 controls included in the penetrance analysis. The Institutional Review Board at the College of Physicians and Surgeons, Columbia University approved this study.

Criteria for Inclusion of Mutation Carriers—All sequence variants identified in cases and controls were genotyped in ethnically matched controls. Sequence variants observed in controls but at a frequency of ≤1% were classified as rare variants. The remaining variants were classified as mutations and included in the analysis based on three criteria: 1) The mutation is absent in controls 2) The mutation is recurrent , has been reported in PD cases (unrelated) in more than one study, or the mutation changes an amino acid that is evolutionarily conserved and which is predicted to effect protein function 3) The mutation is located in the coding region and predicted to change the amino acid sequence.

Molecular Genetic Analysis of Probands

Mutation screening was performed in 247 cases and 105 controls to detect point mutations and exon deletions and duplications in the *Parkin* gene. In a previous study, we sequenced all *Parkin* exons and screened for exon deletions and duplications by semi-quantitative multiplex PCR in 101 cases and 105 controls[18]. One control was subsequently found to have a putative splice variant IVS6-14 C>G, that has not been reported in cases or controls. We consider this a rare variant and not a mutation and have excluded it from the analysis. In this study we report data on an additional 146 PD cases from the GEPD study who were screened for *Parkin* mutations by denaturing high performance liquid chromatography (DHPLC) (WAVE Transgenomic), which has 100% sensitivity and specificity. Primers and DHPLC conditions used for analysis of the *Parkin* gene have been described previously[20]. Amplicons were either directly sequenced (n=126) or analyzed using a *Parkin* genotyping array (n=20)[21] in DNA samples with abnormal elution profiles. The genotyping array has excellent sensitivity and specificity for detection of sequence variants when compared to the gold standard of sequencing[21]. The primers used for PCR amplification of *Parkin* exons 1-12 and intron-exon boundaries and sequencing have been described previously[22]. Cycle sequencing was performed on the purified PCR product as per the manufacturer's instructions (BigDye, Applied Biosystems). Products were analyzed on an ABI3700 genetic analyzer. Chromatograms were viewed using Sequencher (Genecodes) and sequence variants determined. All sequence variants identified in cases and controls were confirmed by analysis in a separate PCR followed by bi-directional sequencing. To identify genomic deletions and exon rearrangements in *Parkin*, semi-quantitative multiplex PCR was performed as previously described[18].

In addition to screening for mutations in *Parkin*, we genotyped five LRRK2 mutations (G2019S, L1114L, I1122V, R1441C and Y1699C) in 247 cases and 104 controls. Results of

the analysis of LRRK2 in all participants in the GEPD study, including both early and late onset PD cases, and all controls have been reported.[23]

Statistical methods

Demographic and clinical characteristics were compared between cases and controls and between mutation carriers and non-carriers. Fisher's exact test for categorical characteristics and Student's t-test for continuous characteristics were used to assess statistical significance. The penetrance of *Parkin* mutations was estimated using the kin-cohort[11] method. In this method, the genotypes of the relatives are first estimated using Mendelian principles and the relationship of the relatives to the proband. Then the observed disease occurrence in the relatives is evaluated in relation to these estimated genotypes. This method assumes that, although the PD case probands were sampled through their AAO, thereby increasing the proportion of carriers among cases and their first-degree relatives, the relatives of these PD cases are representative of randomly chosen individuals with certain genotypes. Hence familial influences on the relatives' PD risk other than the *Parkin* genotype are assumed to be negligible.

First, we computed the probability that each relative carries a mutation. Second, we used the kin-cohort method to estimate genotype-specific disease rates, using the consensus diagnoses of PD in the first-degree relatives[16, 17]. Third, we used Kaplan-Meier survival analysis to compute the cumulative risk of PD in the first-degree relatives of control probands. Kaplan-Meier analysis cannot be directly applied to estimate genotype-specific cumulative incidence in the relatives of cases because the carrier status in these relatives is unknown; however the kin-cohort method allows calculation of cumulative incidence through a method similar to Kaplan–Meier analysis. Confidence intervals for the penetrance and the cumulative incidence were computed using log-log transformation to ensure the lower limits of the confidence intervals were positive [24].

Results

Demographic and Clinical Characteristics of the Case and Control Probands

Demographic and clinical characteristics of the cases and controls are shown in Table 1. The mean AAO of the 247 cases was 41.8 years (SD: 6.8), mean disease duration was 10.7 (SD: 7.6) years, and the total motor score (UPDRS Part III) was 20.3 (SD: 12.8). Nineteen (8.5%) of the 224 cases on whom family history information was available had a diagnosis of PD in a first-degree relative.

Frequency of Parkin Mutations in Cases and Controls

Twenty-five (10.1%) of the 247 cases had a *Parkin* mutation; five (20%) were homozygous, two (8%) were compound heterozygotes and eighteen (72%) were heterozygous (Table 2). Twenty two of the mutations have been previously described in other studies [2, 21, 22] Three new mutations were identified that have not been previously published and were not detected in any of our control samples (Iso298Leu, Asp18Asn, and Pro153Arg). Eleven different point mutations (c.81G>T, Gly319Gly, Arg42Pro, Arg275Trp, Met192Leu, Cys253Tyr, Asp280Asn, Iso298Leu, Arg366Gln, Asp18Asn, Pro153Arg) and four different exon rearrangements were identified (Exon 5 del, Exon 3-4del, Exon 3 40bp del and Exon 2 del) (Table 2). Point mutations included nine missense mutations (Arg42Pro, Arg275Trp, Met192Leu, Cys253Tyr, Asp280Asn, Iso298Leu, Arg366Gln, Asp18Asn, Pro153Arg), one synonymous substitution (Gly319Gly), and a non-coding 5'UTR mutation (c.81G>T). Exon deletions were found in four different exons (exons 2, 3, 4 and 5). 40 percent (10/25) of the variants identified in cases were found in exons encoding functional domains including the ubiquitin domain (Exon 2) and RING1 domain (Exon 7). Six cases carried the Exon 3 40bp

deletion, five cases carried Arg275Trp and two carried Arg42Pro. We previously reported that the synonymous substitution, Leu261Leu, is a variant rather than a disease associated mutation and thus have not included carriers of Leu261Leu in our estimates[23]. Among the 25 carriers, three (12%) had AAO ≤20 (2/3 heterozygotes); four (16%) had AAO 21-30 (3/4 heterozygotes); six (24%) had AAO 31-40 (5/6 heterozgygotes); and twelve (48%) had AAO 41-50 (8/12 heterozgyotes). Among all 247 EOPD case probands, carriers represented 75% (3/4) of those with AAO≤20, 36% (4/11) of those with AAO 21-30, 8% (6/73) of those with AAO 31-40, and 8% (12/159) of those with AAO 41-50.

Clinical Characteristics of Case Probands With and Without Parkin Mutations

Demographic and clinical features of the 25 mutation carriers and 222 non-carriers are shown in Table 3. The AAO of PD was significantly younger in carriers (36.5 ± 10.3) than in non-carriers (42.4 ± 6.1) (p=0.01), but did not differ between heterozygotes compared to compound heterozygotes and homozygotes combined. Other comparisons of clinical features were not significant.

The clinical characteristics of first-degree relatives stratified by the probands' mutation status are presented in Table 4. Information on the family history of PD in first-degree relatives was available for 23/25 carriers and 201/218 non-carriers. One of 23 carrier probands (4.4%) had a family history of PD. This proband is homozygous for an 40 bp deletion in exon 3 and had two affected siblings (AAO 26, 30).

Penetrance Estimates of Parkin Mutations

The probability of a relative being a carrier, whether or not he/she was actually diagnosed with PD, stratified by the proband's carrier status is presented in Table 5. In the calculation, the population frequency of *Parkin* mutations, p, was assumed to be 0.03% {Lucking, 2000 #1168; Oliveira, 2003 #2;. We have run a sensitivity analysis by taking p to be 2.8% (the upper limit of the 95% exact confidence interval of the mutation frequency estimated from the controls) and the results did not change, suggesting that the estimates are robust to misspecification to the population frequency of *Parkin* mutations.

The expected genotype distribution in the relatives and the prevalence of a history of PD in relatives predicted to be carriers or noncarriers are shown in Table 6. To obtain these prevalence estimates, we first computed the probability that each of the relatives was a carrier based on the observed carrier status in the probands (see Table 1) and then combined the information on the predicted genotypes in the relatives with the observed PD diagnoses in the relatives. There were 93 relatives expected to be carriers (homozygotes or heterozygotes), among whom 2 had PD (Table 6). Therefore, the prevalence of a history of PD in carrier relatives was estimated at 2/93 [2.2% (CI: 0.3%, 7.6%)]. Among relatives expected to be noncarriers (N=1237), 19 had PD (Table 6); thus the prevalence of a history of PD in noncarrier relatives was estimated as 1.7% (0.9-2.4%). These two prevalence estimates did not differ significantly.

The cumulative incidence of PD in 1330 relatives of case probands (without regard to carrier status) was estimated to be 1.7% (95% CI: 0.9-3.3%) to age 65, and 5.9% (95% CI: 3.7-9.3%) to age 80. Estimates of the cumulative risk of PD in *Parkin* carriers and non-carriers and the cumulative risk of PD in 647 relatives of controls are presented in table 7. Among relatives expected to be carriers, the cumulative incidence of PD was 7.0% to age 65 and remained so up to age 80. Among relatives expected to be noncarriers, the cumulative incidence was 1.7% to age 65 and 6.1% to age 80. The ratio of the cumulative incidence to age 80 for carriers versus non-carriers was 1.1 (95% CI: 0.07-18.8). Estimates of risk in carriers and noncarriers did not differ significantly with our sample size. The cumulative

incidence in relatives of controls was similar to that in relatives expected to be noncarriers (1.1% to age 65 and 4.1% to age 80).

We have reported that LRRK2 mutations may be responsible for familial aggregation in both EOPD and LOPD in the GEPD study{Clark, 2006 #12300}. To separate out the effect of the LRRK2 G2019S mutation (the only observed LRRK2 mutation in the cases), we repeated our analyses using 214 case probands who did not carry a LRRK2 mutation. However, we can not we can not exclude the possibility that they carry 'other' mutations in the LRRK2 gene as we did not completely sequence the LRRK2 gene but only genotyped 5 previously reported LRRK2 mutations. There were 1274 relatives included in the analysis. Since these relatives were family members of probands who did not carry any of the five previously identified LRRK2 mutations, it is unlikely they carry any of these mutations. The cumulative incidence to age 80 for relatives expected to be non-carriers of either LRRK2 mutations or *Parkin* mutations was 5.9%, compared to 6.1% for relatives expected to be noncarriers of *Parkin* mutations. The cumulative incidence of PD to age 80 in non-*Parkin*, non-LRRK2 carrier relatives was not significantly different from controls.

Discussion

Using the kin-cohort method, we have shown that the cumulative risk to age 65 in a relative of an EOPD case who is not estimated to carry a *Parkin* mutation is not significantly greater than the general population risk among controls. We estimated a cumulative risk of PD in carriers of Parkin mutations (age-specific penetrance) of 7.0% (95% CI: 0.4-71.9%) up to age 80. We were unable to examine risk of PD among heterozygotes separately. *Parkin* heterozgyosity may not be sufficient for the development of PD. A recent paper suggested that *Parkin* variants were equally common in cases and controls in predominantly non-coding regions[25].

The sampling scheme of our study, in which probands were ascertained without regard to their family history of PD, differs markedly from that in other studies that included only families with multiple affected individuals. As noted in the context of estimating the penetrance of mutations in BRCA1 and BRCA2, penetrance estimates are inflated when based on samples selected though family history [12, 15], and hence we believe our estimates are more representative of those in the general population than are those derived from familial samples. While the confidence intervals are extremely wide, the observed low frequency of PD in first-degree relatives of *Parkin* mutation carriers, 72% of whom are heterozygotes, may be a reflection of the low penetrance in carriers.

The frequency of *Parkin* mutations was 10.1% (95% CI: 8.0-16.4%) in 247 cases with AAO ≤ 50, which is within the range of other series with primarily sporadic cases (15-18%)[6], or population-based series (9%)[26]. The mean AAO of our case sample was 41.6 years, and 48% of the cases had AAO between 40 and 50. The frequency of *Parkin* mutations has been reported to decrease with increasing age-at-onset[3, 6]. While the age-specific frequencies of carriers are similar to other reported series[6], the high percentage of older cases in this study may explain why we report a frequency at the lower end of the spectrum.

Limitations

There are three important limitations in this study. Most importantly, the number of relatives of probands who are estimated to carry *Parkin* mutations is limited, which results in cumulative risk estimates with wide confidence intervals. We have limited our study to only those who participated in the GEPD study for whom we have accurate information on vital status, a necessary criterion for the kin-cohort method. In addition, only 105 controls were completely sequenced for the *Parkin* gene, which may explain our inability to detect

differences between relatives of PD probands and relatives of control probands. The sample size required for a typical kin-cohort study is usually large, due to the low population prevalence of the mutation being studied. Second, the diagnosis in the relatives was a best estimate diagnosis. We have previously reported high sensitivity (95.5%) and specificity (96.2%) of our family history questionnaire based on examination of 141 relatives. Third, we did not have actual genotype data on relatives. We used the kin-cohort method to estimate penetrance in the absence of these data.

Some relatives may have been too young to manifest PD. Our sampling plan was to include all probands with AAO ≤50 regardless of their family history of PD and AAO in the relatives. The relatives of these probands are likely to be younger than the relatives of randomly selected PD patients. In a study of affected sibling pairs, the mean AAO of heterozygous *Parkin* carriers was reported to be 49.6.[10] Forty-nine percent of the relatives in our sample were younger than 49.6 (Table 4). Our estimates of the prevalence of a history of PD in the relatives do not account for this young age distribution, and thus underestimate prevalence of PD according to *Parkin* genotype in the general population. We accounted for the younger age distribution by computing age-specific cumulative incidence of PD according to genotype.

One assumption required for the kin-cohort analyses to be valid is that risk of PD in relatives within the same family is independent, given the proband's *Parkin* genotype. The presence of other genetic and environmental risk factors that aggregate in the families may violate this assumption and bias the penetrance estimation[15, 27]. Due to the possibility of additional unspecified familial risk factors in the relatives of early-onset PD probands, penetrance estimates obtained from our sample can be applied to the population of relatives of the early-onset PD probands but not to the entire population.

We are currently recruiting a larger multi-center sample of early-onset cases and examining and obtaining DNA in relatives of carrier probands. We hope to use this new sample to refine our estimates of penetrance in heterozygous, homozygous, and compound heterozygous carriers.

Acknowledgments

This study was funded by NIH NS36630, RR00645 (KM) and the Parkinson's Disease Foundation (KM and LNC). The authors thank Dr. Paul Greene for his participation.

References

1. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–8. [PubMed: 9560156]
2. Hedrich K, Eskelson C, Wilmot B, et al. Distribution, type, and origin of Parkin mutations: Review and case studies. Mov Disord 2004;19:1146–57. [PubMed: 15390068]
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;342:1560–7. [PubMed: 10824074]
4. Hedrich K, Marder K, Harris J, et al. Evaluation of 50 probands with early-onset Parkinson's disease for Parkin mutations. Neurology 2002;58:1239–46. [PubMed: 11971093]
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;8:567–74. [PubMed: 10072423]
6. Periquet M, Latouche M, Lohmann E, et al. Parkin mutations are frequent in patients with isolated early-onset parkinsonism. Brain 2003;126:1271–8. [PubMed: 12764050]

7. Lohmann E, Periquet M, Bonifati V, et al. How much phenotypic variation can be attributed to parkin genotype? Ann Neurol 2003;54:176–85. [PubMed: 12891670]
8. Foroud T, Uniacke SK, Liu L, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. Neurology 2003;60:796–801. [PubMed: 12629236]
9. Oliveira SA, Scott WK, Martin ER, et al. Parkin mutations and susceptibility alleles in late-onset Parkinson's disease. Ann Neurol 2003;53:624–9. [PubMed: 12730996]
10. Sun M, Latourelle JC, Wooten GF, et al. Influence of heterozygosity for parkin mutation on onset age in familial Parkinson disease: the GenePD study. Arch Neurol 2006;63:826–32. [PubMed: 16769863]
11. Wacholder S, Hartge P, Struewing JP, et al. The kin-cohort study for estimating penetrance. Am J Epidemiol 1998;148:623–30. [PubMed: 9778168]
12. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401–8. [PubMed: 9145676]
13. Dekker MC, Bonifati V, Van Duijn CM. Parkinson's disease: piecing together a genetic jigsaw. Brain. 2003
14. Marder K, Levy G, Louis ED, et al. Familial aggregation of early- and late-onset Parkinson's disease. Ann Neurol 2003;54:507–13. [PubMed: 14520664]
15. Gail MH, Pee D, Benichou J, Carroll R. Designing studies to estimate the penetrance of an identified autosomal dominant mutation: cohort, case-control, and genotyped-proband designs. Genet Epidemiol 1999;16:15–39. [PubMed: 9915565]
16. Marder K, Levy G, Louis ED, et al. Accuracy of family history data on Parkinson's disease. Neurology 2003;61:18–23. [PubMed: 12847150]
17. Levy G, Louis ED, Mejia-Santana H, et al. Lack of familial aggregation of Parkinson disease and Alzheimer disease. Arch Neurol 2004;61:1033–9. [PubMed: 15262733]
18. Clark LN, Afridi S, Karlins E, et al. Case-control study of the parkin gene in early-onset Parkinson disease. Arch Neurol 2006;63:548–52. [PubMed: 16606767]
19. Fahn S, Marsden CD, Calne D. Recent Developments in Parkinson's Disease. Florham Park, N. J. Macmillan Healthcare Information. 1987
20. Pigullo S, De Luca A, Barone P, et al. Mutational analysis of parkin gene by denaturing high-performance liquid chromatography (DHPLC) in essential tremor. Parkinsonism Relat Disord 2004;10:357–62. [PubMed: 15261877]
21. Clark LN, Haamer E, Mejia-Santana H, et al. Construction and validation of a Parkinson's disease mutation genotyping array for the Parkin gene. Mov Disord 2007;22:932–7. [PubMed: 17415800]
22. West A, Periquet M, Lincoln S, et al. Complex relationship between Parkin mutations and Parkinson disease. Am J Med Genet 2002;114:584–91. [PubMed: 12116199]
23. Clark LN, Wang Y, Karlins E, et al. Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. Neurology 2006;67:1786–91. [PubMed: 17050822]
24. Kalbfleisch JDaP, RL. The Statistical Analysis of Failure Time Data. John Wiley & Sons, Inc.; New York: 1980.
25. 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. [PubMed: 17187375]
26. Kann M, Jacobs H, Mohrmann K, et al. Role of parkin mutations in 111 community-based patients with early-onset parkinsonism. Ann Neurol 2002;51:621–5. [PubMed: 12112109]
27. Begg CB. On the use of familial aggregation in population-based case probands for calculating penetrance. J Natl Cancer Inst 2002;94:1221–6. [PubMed: 12189225]

Table 1

Demographic Characteristics of Case and Control Probands

	Total (n=351)	Cases (n=247)	Controls (n=104)	Significance Cases vs. Controls
% male (n)	59.7 (209)	61.1 (151)	55.8 (58)	0.40
Age (years) (sd)	54.9 (10.2)	52.5 (9.0)	60.8 (10.5)	<0.001
% White (n)	84.3 (296)	81.0 (200)	92.3 (96)	
% African-American (n)	2.3 (8)	2.0 (5)	2.9 (3)	
% Hispanic (n)	8.0 (28)	10.1 (25)	2.9 (3)	
% Other (n)	5.4 (19)	6.9 (17)	1.9 (2)	0.02
Years Education (sd)	15.5 (3.2)	15.5 (3.3)	15.5 (2.9)	0.97
% with *Parkin* variants (n)	7.1 (25)	10.1 (25)	0.0 (0)	<0.001
% with family history of PD* (n)	7.7 (25)	8.5 (19/224)	5.8 (6/103)	0.50

*Family history was available for 328 probands (224 cases and 103 controls)

Table 2

Parkin Mutations identified in cases

Patient	Exon	Mutation	Zygosity	AAO (years)	Ethnicity
1	2	Arg42Pro	Het	44	White/non Hispanic
2	2	Arg42Pro	Het	32	Hispanic
3	2	Exon 2 deletion	Hom	42	White/non Hispanic
4	2	Asp18Asn	Het	32	Hispanic
5	3	Exon 3 40bp deletion	Hom	38	White/non Hispanic
6	3,7	Exon 3 40bp deletion + Arg275Trp	Comp. Het	19	White/non Hispanic
7	3	Exon 3 40bp deletion	Het	29	White/non Hispanic
8	3	Exon 3 40bp deletion	Hom	47	White/non Hispanic
9	3	Exon 3 40bp deletion	Hom	45	White/non Hispanic
10	3	Exon 3 40bp deletion	Hom	27	White/non Hispanic
11	3,4	Exon 3-4 deletion	Het	16	Hispanic
12	4	Pro153Arg	Het	42	Hispanic
13	5	Exon 5 deletion	Het	48	White/non Hispanic
14	5	Exon 5 deletion	Het	50	White/non Hispanic
15	5	Met192Leu	Het	36	Hispanic
16	5	Met192Leu.	Het	42	Hispanic
17	7	Cys253Tyr +Asp280Asn	Comp. Het	47	White/non Hispanic
18	5'UTR	c.81G>T	Het	28	Hispanic
19	7	Arg275Trp	Het	47	White/non Hispanic
20	7	Arg275Trp	Het	24	Other
21	7	Arg275Trp	Het	35	White/non Hispanic
22	7	Arg275Trp	Het	42	Hispanic
23	8	Iso298Leu[1]	Het	37	White/non Hispanic
24	9	Gly319Gly	Het	47	Hispanic
25	10	Arg366Gln	Het	17	White/non Hispanic

Table 3

Characteristics of Case Probands With and Without *Parkin* Mutations

	Mutation (n=25)	No mutation (n=222)	*p* value
Current age (sd)	47.2 (9.9)	53.1 (8.7)	0.008
Age onset (sd)	36.5 (10.3)	42.4 (6.1)	0.01
% male (n)	40.0 (10)	63.5 (141)	0.03
% Ethnicity: White (n)	64.0 (16)	82.3 (184)	
African-American (n)	0.0 (0)	2.3 (5)	
Hispanic (n)	28.0 (7)	8.1 (18)	0.03
Other (n)	8.0 (2)	6.8 (15)	
Years education (sd)	14.1 (3.8)	15.6 (3.3)	0.06
Duration PD (yrs)(sd)	10.6 (7.5)	10.7 (7.7)	0.96
Total motor score (UPDRS III)(sd)	22.1 (15.8)	20.1 (12.2)	0.57
Total modified mini mental state (mMMS) (sd)	52.3 (5.3)	53.7 (4.6)	0.24
% Family history PD in first-degree relative*	4.4 (1/23)	8.9 (18/201)	0.72
% Bradykinesia	88.0 (22)	82.9 (184)	0.78
% Rest tremor	80.0 (20)	78.2 (172/220)	1.00
% Rigidity	96.0 (24)	98.7 (219)	0.35
% Postural instability	44.0 (11)	45.0 (99/220)	1.00
% Asymmetry	96.0 (24)	88.5 (192/217)	0.49
% on L dopa	70.8 (17/24)	70.2 (153/218)	1.00
% Improve L dopa	95.0 (19/20)	97.4 (150/154)	0.46
%On-off	51.7 (15)	53.6 (113/211)	1.00
Hoehn and Yahr (sd)	2.3 (0.9)	2.1 (0.9)	0.32
% First symptom†: rest tremor (n)	44.0 (11)	48.9 (108)	0.68
% First symptom: gait (n)	0.0	4.5 (10)	0.60
% First symptom: rigidity (n)	8.0 (2)	9.5 (21)	1.00
% First symptom: bradykinesia (n)	4.0 (1)	4.1 (9)	1.00
% First symptom: motor (n)	4.0 (1)	1.8 (4)	0.42
% First symptom: poor balance (n)	4.0 (1)	0.5 (1)	0.19
% First symptom: pain (n)	8.0 (2)	1.8 (4)	0.12

*Family history was available for 224 case probands.

†First symptom information was available for 221 out of 222 non-carriers.

Table 4

Characteristics of first-degree relatives of case probands stratified by probands' carrier status

	Total Relatives of Case Probands (n=1330)	Relatives of Homozyogous/ Compound Heterozygous Carriers (n=42)	*Relatives of Heterozygous carriers* (n=110)	*Relatives of Non-carriers* (n=1178)	*Relatives of Control Probands* (n=638)	*Significance across probands' genotypes*
% male (n)	47.7 (634)	52.4 (22)	44.6 (49)	47.8 (563)	50.9 (325)	0.09
Age (years) (sd)	49.7 (22.7)	47.6 (22.2)	45.4 (19.7)	50.2 (22.9)	53.9 (20.2)	0.08
Parent (years, sd)	73.0 (14.0)	72.8 (9.1)	65.8 (13.7)	73.6 (13.9)	72.6 (13.9)	0.01
Siblings (years, sd)	49.6 (11.6)	46.7 (8.8)	45.3 (12.1)	50.2 (11.6)	55.1 (14.4)	0.01
Offspring (years, sd)	24.9 (11.5)	19.3 (8.8)	22.8 (9.0)	25.2 (11.7)	33.6 (11.1)	0.14
Ethnicity (%, n)						
White	74.4 (989)	100 (42)	42.7 (47)	76.4 (900)	91.5 (584)	
African-American	2.9 (39)	0	0	3.3 (39)	1.7 (11)	<0.001
Hispanic	13.9 (185)	0	47.3 (52)	11.3 (133)	3.6 (23)	
Other	8.8 (117)	0	9.4 (11)	9.0 (106)	3.1 (20)	
Years Education (sd)	12.6 (4.9)	13.4 (4.4)	11.7(4.9)	12.7 (4.9)	13.3 (4.1)	0.11
Relationship %,(n)						
Parents	32.4 (431)	30.9 (13)	28.2 (31)	32.9 (387)	31.5 (201)	
Siblings	37.4 (497)	42.9 (18)	46.4 (51)	36.3 (428)	36.7 (234)	0.29
Offspring	30.2 (402)	26.2 (11)	25.5 (28)	30.8 (363)	31.8 (203)	
Number Affected (%, n)						
All relatives	1.6 (21)	4.8 (2)	0	1.6 (19)	0.9 (6)	0.09
AAO years for affecteds mean, (sd)	61.3 (16.1)	27.0 (2.8)	--	64.9 (11.9)	66.5 (12.8)	<0.001
Parents	3.5 (15)	0	0	3.9 (15)	3.0 (6)	0.51
AAO years for affecteds mean, (sd)	69.1 (7.3)	--	--	69.5 (7.7)	66.5 (12.8)	0.60
Siblings % (n)	1.2 (6)	7.7 (2)	0	1.0 (4)	0	0.07
AAO years for affecteds mean, (sd)	41.7 (15.3)	27.0 (2.8)	--	49.0 (13.2)	--	0.09
Offspring	0	0	0	0	0	

Table 5

Probability of a relative being a carrier stratified by the proband's mutation status*

Relatives of homozygous or compound heterozygous probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	P	$1-p$	0
Sibling	$1/4*(p^2-2p-1)$	$1/2*(-p^2-1)$	$1/4*(1-p)^2$
Offspring	P	$1-p$	0

Relatives of heterozygous probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	$1/2*p$	1/2	$1/2*(1-p)$
Sibling	$1/4*(p^2+p)$	$1/2*(-p^2-p-1)$	$1/4*(p^2-3p+2)$
Offspring	$1/2*p$	1/2	$1/2*(1-p)$

Relatives of non-carrier probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	0	p	$1-p$
Sibling	$1/4*p^2$	$1/2(-p^2+2p)$	$1/4*(p^2-4p+4)$
Offspring	0	p	$1-p$

* p is the population frequency of the mutation.

Table 6

Estimated genotype distribution and prevalence of a history of PD in relatives of cases

	Carriers (Homozygotes and Heterozygotes)		Non-carriers		Total
	PD	Non-PD	PD	Non-PD	
Parent	0	29	15	387	431
Siblings	2	38	4	453	497
Offspring	0	25	0	377	402
Total	2	91	19	1218	1330
Prevalence (exact 95% CI)	2.2% (0.3%, 7.6%)		1.7% (0.9%, 2.4%)		

Table7

Estimated cumulative incidence of PD in *Parkin* carrier relatives, non-carrier relatives, and relatives of controls

Age	*Parkin* Carrier (Homozygotes and heterozygotes) Relatives (95% CI)	Non-carrier Relatives (95% CI)	Relatives of Controls (95% CI)
25	3.7% (1.1, 84.0%)	0	0
35	7.0% (0.4,71.9%)	0	0
45	7.0% (0.4,71.9%)	0.2% (0.1, 1.0%)	0.2% (0.03-1.7%)
55	7.0% (0.4,71.9%)	0.4 (0.1, 1.4%)	0.2% (0.03, 1.7%)
60	7.0% (0.4,71.9%)	1.1 (0.5, 2.5%)	0.6% (0.1, 2.5%)
65	7.0% (0.4,71.9%)	1.7% (0.8, 3.4%)	1.1% (0.3, 3.4%)
70	7.0% (0.4,71.9%)	3.3% (1.9, 5.7%)	1.1% (0.3, 3.4%)
75	7.0% (0.4,71.9%)	4.6% (2.7, 7.5%)	2.8% (1.1, 7.2%)
80	7.0% (0.4,71.9%)	6.1% (3.8, 9.8%)	4.1% (1.7, 9.8%)

ORIGINAL CONTRIBUTION

Complete Genomic Screen in Parkinson Disease

Evidence for Multiple Genes

William K. Scott, PhD; Martha A. Nance, MD; Ray L. Watts, MD; Jean P. Hubble, MD; William C. Koller, MD; Kelly Lyons, PhD; Rajesh Pahwa, MD; Matthew B. Stern, MD; Amy Colcher, MD; Bradley C. Hiner, MD; Joseph Jankovic, MD; William G. Ondo, MD; Fred H. Allen, Jr, MD; Christopher G. Goetz, MD; Gary W. Small, MD; Donna Masterman, MD; Frank Mastaglia, MD; Nigel G. Laing, MD; Jeffrey M. Stajich, PA-C; Brandon Slotterbeck, BS; Michael W. Booze, BS; Robert C. Ribble, BS; Evadnie Rampersaud, MSPH; Sandra G. West, BS; Rachel A. Gibson, PhD; Lefkos T. Middleton, MD; Allen D. Roses, MD; Jonathan L. Haines, PhD; Burton L. Scott, PhD, MD; Jeffery M. Vance, PhD, MD; Margaret A. Pericak-Vance, PhD

Context The relative contribution of genes vs environment in idiopathic Parkinson disease (PD) is controversial. Although genetic studies have identified 2 genes in which mutations cause rare single-gene variants of PD and observational studies have suggested a genetic component, twin studies have suggested that little genetic contribution exists in the common forms of PD.

Objective To identify genetic risk factors for idiopathic PD.

Design, Setting, and Participants Genetic linkage study conducted 1995-2000 in which a complete genomic screen (n=344 markers) was performed in 174 families with multiple individuals diagnosed as having idiopathic PD, identified through probands in 13 clinic populations in the continental United States and Australia. A total of 870 family members were studied: 378 diagnosed as having PD, 379 unaffected by PD, and 113 with unclear status.

Main Outcome Measures Logarithm of odds (lod) scores generated from parametric and nonparametric genetic linkage analysis.

Results Two-point parametric maximum parametric lod score (MLOD) and multipoint nonparametric lod score (LOD) linkage analysis detected significant evidence for linkage to 5 distinct chromosomal regions: chromosome 6 in the parkin gene (MLOD=5.07; LOD=5.47) in families with at least 1 individual with PD onset at younger than 40 years, chromosomes 17q (MLOD=2.28; LOD=2.62), 8p (MLOD=2.01; LOD=2.22), and 5q (MLOD=2.39; LOD=1.50) overall and in families with late-onset PD, and chromosome 9q (MLOD=1.52; LOD=2.59) in families with both levodopa-responsive and levodopa-nonresponsive patients.

Conclusions Our data suggest that the parkin gene is important in early-onset PD and that multiple genetic factors may be important in the development of idiopathic late-onset PD.

JAMA. 2001;286:2239-2244 www.jama.com

PARKINSON DISEASE (PD) IS A NEURODEGENERATIVE disease that affects more than a half-million people in the United States.[1] The economic, social, and emotional burden of PD will increase as the population ages. Controversy has surrounded the etiology of PD, with evidence suggesting that both genetic and environmental factors influence disease risk. Familial aggregation of PD has been observed for decades.[2] Data from family studies, including a recent large study from Iceland,[3] have shown that the sibling recurrence risk ratio ranges from 2 to 10, suggesting that a genetic component to PD exists. However, twin studies[4-6] have produced conflicting results about the genetic contributions, suggesting that little if any genetic contribution exists in the development of PD.

See also pp 2245 and 2324.

Previous efforts to identify genetic risk factors for PD have focused primarily on rare, simple autosomal dominant and recessive forms of the disease. Mutations in the α-synuclein gene, located on chromosome 4q, have been shown to be rare causes of autosomal dominant, early-onset PD.[7] Mutations in the parkin gene, located on chromosome 6q, have been reported in fami-

Author Affiliations are listed at the end of this article.
Corresponding Author and Reprints: Margaret A. Pericak-Vance, PhD, Center for Human Genetics, Box 3445, Duke University Medical Center, Durham, NC 27710 (e-mail: mpv@chg.mc.duke.edu).

lies with rare autosomal recessive juvenile parkinsonism and autosomal recessive early-onset PD.[8,9] Linkage of several large families with autosomal dominant PD to chromosome 2 has been reported,[10] but a disease-causing gene remains to be identified. Collectively, these studies have demonstrated genetic effects only for rare single-gene variants of PD. To examine the broader issue of genetic effects in idiopathic PD, we performed a complete genomic screen for linkage analysis in 174 families with PD containing at least 1 affected relative pair.

METHODS

Family Data

In 1995-2000, we coordinated a 13-center effort selecting multiplex (≥2 individuals diagnosed as having PD) families for genetic studies of PD. History of PD was documented for each family by conducting a standard interview with the proband or a knowledgable family informant. Diagnostic and exclusion criteria, based on previously published diagnostic criteria for PD,[11-13] were adopted by all participating clinicians before beginning selection of families.

All participants were examined before enrollment in the study by a board-certified neurologist or a physician assistant trained in neurologic disease and supervised by a neurologist. Participants were classified as affected, unclear, or unaffected based on neurologic examination findings and clinical history. Affected individuals had at least 2 cardinal signs of PD (eg, rest tremor, bradykinesia, and rigidity) and no atypical clinical features or other causes of parkinsonism. Individuals with unclear status had only 1 sign of PD, a history of atypical clinical features, or both. Unaffected individuals had no signs of PD. Excluded from participation were individuals with a history of encephalitis, neuroleptic therapy within the year before diagnosis, evidence of normal pressure hydrocephalus, or a clinical course with unusual features suggestive of atypical or secondary parkinsonism.

Age at onset of PD was self-reported and defined as the age at which the affected individual could first recall noticing one of the primary signs of PD. Because a positive response to levodopa therapy was considered supportive of a diagnosis of idiopathic PD, physician and patient observations of whether symptoms of PD were significantly improved by levodopa therapy were used to classify individuals as responsive or nonresponsive to levodopa.[12,13] Individuals for whom levodopa was of uncertain benefit or who never received levodopa therapy were classified as having unknown levodopa response. Within-family variation in response to levodopa was considered a marker of potential phenotypic and thus genotypic heterogeneity.

To ensure diagnostic consistency across sites, clinical data for all participants were reviewed by a clinical adjudication board, which consisted of a board-certified neurologist with fellowship training in movement disorders (B.L.S.), a dually board-certified neurologist and medical geneticist (J.M.V.), and a certified physician assistant (J.M.S.). Forms with missing data or data inconsistent with the diagnosis assigned to the individual were referred back to the collaborating site for clarification. All participants gave written informed consent before venipuncture and data collection according to protocols approved by each center's institutional review board.

DNA Analysis

Genomic DNA was extracted from whole blood using the Puregene system (Gentra Systems, Minneapolis, Minn). Analysis was performed on 344 microsatellite markers with an average spacing of 10 cM (centimorgans). Genotyping was performed by the FAAST method.[14] All samples in the laboratory were identified only by a sequential 6-digit identification number, which was given to the sample when it was received in the DNA-Bank. No names or family relationships were provided to any laboratory technician.

Systematic genotyping errors were minimized using a system of quality control checks with duplicated samples. For each 96-well polymerase chain reaction plate, 2 standard samples from Centre d'Etude du Polymorphisme Humain (Paris, France) families were included and 6 samples were duplicated either on that plate or another plate in the screen. Laboratory technicians not involved in the determination of genotypes performed the placement of these duplicated quality control samples. Thus, the laboratory technicians who read the genotypes were blinded to the location of the matching partner for each quality control sample to avoid bias in interpretation of results. Statistical analysts used automated computer scripts to check each set of genotypes submitted by the technician for mismatches between the duplicated samples; mismatches are indicative of potential genotype reading errors, misloading of samples, and sample mix-ups. These mismatches were then sent as one of a large group of surrounding genotypes for rechecking. Thus, the technician had no knowledge of the actual genotype in question. As an additional quality control measure, potential pedigree errors were checked using the program RELPAIR,[15] which infers likely relationships between pairs of relatives using identical by descent–sharing estimates from a set of microsatellite markers.

Statistical Analysis

Data analysis used a multianalytical approach consisting of both parametric lod score and nonparametric affected relative pair methods. Maximum parametric lod scores (MLODs) for each marker were calculated using the VITESSE and HOMOG program packages.[16,17]

The MLOD is the lod score maximized over the 2 genetic models tested, allowing for genetic heterogeneity. Dominant and recessive low-penetrance (affecteds-only) models were considered. Only individuals with a clear diagnosis of PD were considered affected in these analyses. Estimates of

prevalence of PD range from 0.3% in individuals 40 years or older to 2.5% in individuals 70 years or older.[1] Based on these prevalence estimates and allowing for age-dependent or incomplete penetrance, disease allele frequencies of 0.001 for the dominant model and 0.20 for the recessive model were used. Marker allele frequencies were generated from more than 150 unrelated white individuals.

Multipoint nonparametric lod scores (LODs) were calculated using GENEHUNTER-PLUS.[18] Sex-averaged intermarker distances from the Marshfield Center for Medical Genetics, Marshfield, Wis, genetic linkage maps (http://www.marshfieldclinic.org/research/genetics/map_markers/maps/indexmapframes.html) were used in these analyses. In contrast to nonparametric linkage approaches that consider allele sharing in pairs of affected siblings,[19] GENEHUNTER-PLUS considers allele sharing across pairs of affected relatives (or all affected relatives in a family) in moderately sized pedigrees. We selected this program because of the additional power contributed to the sample by the 75 affected relative pairs that would be excluded by an affected sibpair analysis. Because of computational constraints on pedigree size, 27 unaffected individuals from 12 families were omitted from this analysis.

Because of the potential genetic heterogeneity in this sample, a priori we stratified the data set into 3 subsets. The early-onset PD subset included 18 families with at least 1 member with early-onset PD (<40 years[20]) (range, 12-66 years). The levodopa-nonresponsive subset included 9 families with late-onset PD that contained at least 1 affected individual who was determined to be nonresponsive to levodopa therapy. The late-onset idiopathic PD subset contained 147 families with late-onset PD.

Traditionally in linkage analysis (particularly of mendelian traits), a LOD of more than 3 (corresponding to 1000:1 odds in favor of linkage) is considered strong evidence for linkage. However, such a threshold is likely too stringent for initial efforts to find complex disease genes; using a lower threshold (such as a LOD of >1) in an initial genomic screen may help ensure that genes with modest effects are not missed.[21] Recent genomic screens have used reduced thresholds for declaring results "interesting" for further study.[22,23] In this study, only regions generating both MLODs and LODs of more than 1.5 were classified as having interesting results. Although this approach may increase the number of false-positive results that are subjected to more detailed examination, it decreases the more serious possibility of missing a true genetic effect.

Table. Regions Generating 2-Point MLODs and Multipoint LODs Greater Than 1.5*

			2-Point MLOD		Multipoint LOD	
Chromosome	Set	Peak Marker	MLOD	Location, cM†	Peak LOD	Location, cM†
3q	LDNR	*D3S2460*	1.62	135	1.54	134
5q	Overall	*D5S816*	2.39	139	1.50	139
6q	EOPD	*D6S305*	5.07	166	5.47	166
8p	Overall	*D8S520*	2.01	21	2.22	27
	LOPD	*D8S520*	1.92	21	1.69	27
9q	LDNR	*D9S301*	1.52	66	2.59	140
17q	Overall	*D17S921*	1.92	36	2.02	56
	LOPD	*D17S1293*	2.28	56	2.62	56

*MLOD indicates maximum parametric lod score; LOD, nonparametric lod score; LDNR, levodopa nonresponsive; EOPD, early-onset Parkinson disease; and LOPD, late-onset Parkinson disease.
†Location is presented in centimorgans (cM) from the p telomere, based on the Marshfield Clinic sex-averaged maps.

RESULTS

All individuals potentially informative for linkage were considered for selection in each family, and all family members sampled at the time of the study were included in this analysis. The families contained an average of 2.3 affected individuals and an average of 1.5 affected relative pairs per family. Although most affected relative pairs were affected sibpairs (185/260), there were 75 other affected relative pairs (19 avuncular, 51 cousin, and 5 parent-child pairs) in the data set, indicating that families were often multigenerational in structure and that the study was not limited to affected sibpairs.

All families studied were white and included 870 individuals (an average of 5 per family). Of these individuals, 378 (43%) were diagnosed as having PD, 379 (44%) were unaffected, and 113 (13%) had unclear status. In affected individuals, the mean (SD) age at onset of PD was 59.9 (12.6) years (range, 12-90 years), and the mean (SD) age at examination was 69.9 (10.2) years (range, 33-90 years). Mean (SD) age at examination in unaffected individuals was 67.1 (12.9) years (range, 31-96 years), and mean (SD) age at examination in those with unclear PD status was 72.1 (11.6) years (range, 49-90 years). Mean age at onset in the families with early-onset PD was 39.7 years (range, 12-66 years), whereas mean age at onset in the families with late-onset PD was 62.7 years (range, 40-90 years). The 2 age-of-onset groups were similar with respect to average family size and structure.

Analysis of the clinical parameters of the collected data set did not differ significantly from the patient data collected for other studies in which the focus was on the enrollment of patients with PD for clinical trials.[24] Genetic regions generating both MLODs and LODs of greater than 1.5 are listed in the TABLE. Markers on chromosomes 5q, 8p, and 17q generated interesting 2-point and multipoint lod scores (MLODs and LODs of more than 1.5) in the overall sample of 174 families (FIGURE 1). The strongest evidence for linkage in the overall data set was on chromosome 8p (MLOD = 2.01 at *D8S520*; LOD = 2.22). Other regions with interesting 2-point and multipoint results were 5q (MLOD = 2.39

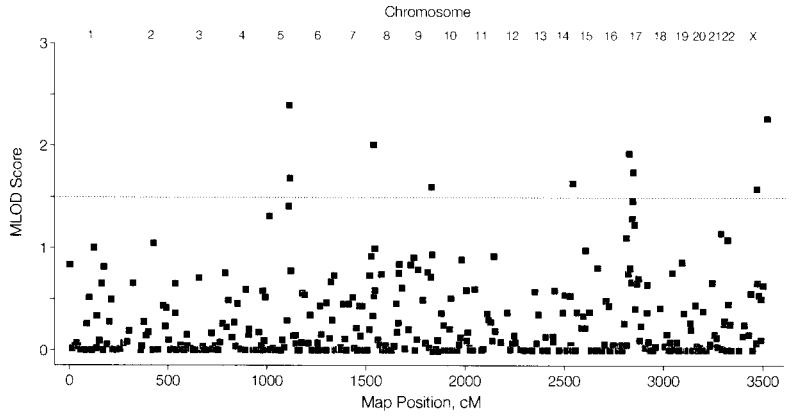
at *D5S816*; LOD = 1.5) and 17q (MLOD = 1.92 at *D17S921*; LOD = 2.02).

FIGURE 2 presents the 2-point MLOD results for the data stratified by age at onset and levodopa response. In the subset of 18 families with early-onset PD, a significant MLOD was obtained at *D6S305*, located in intron 7 of the parkin gene (MLOD = 5.07). Multipoint analysis confirmed these findings, resulting in a LOD of 5.47. No other regions of the genome generated both MLODs and LODs >1.5 in the early-onset subset.

The strongest linkage result obtained in the subset containing 147 families with late-onset idiopathic PD was on 17q (MLOD = 2.28 at *D17S1293*; LOD = 2.62). A second region of interest was located on 8p (MLOD = 1.92 at *D8S520*; LOD = 1.69).

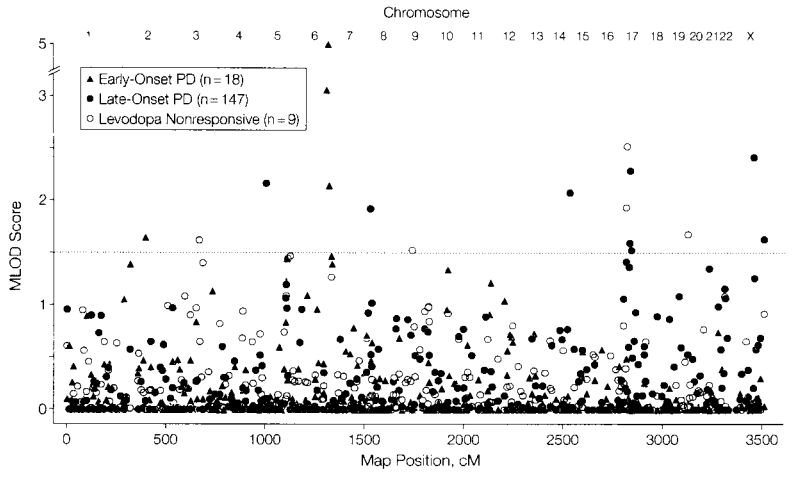
The 9 levodopa-nonresponsive families produced novel results on chromosome 9q (MLOD = 1.52 at *D9S301*; LOD = 2.59). An additional region of interest was detected on 3q (MLOD = 1.62 at *D3S2460*; LOD = 1.54).

Figure 1. Two-Point Maximum Parametric Lod Scores (MLODs) for the Overall Data Set (n = 174)



Each square represents the MLOD at 1 of the 344 markers analyzed in the genomic screen. Regions of interest (see the "Methods" section) are located above the dotted horizontal line (MLOD >1.5). cM indicates centimorgans.

Figure 2. Two-Point Maximum Parametric Lod Scores (MLODs) Stratified by Age at Onset of PD and Levodopa Response



Each point represents the MLOD at 1 of the 344 markers analyzed in each subset. Regions of interest (see the "Methods" section) are located above the dotted horizontal line (MLOD >1.5). cM indicates centimorgans; PD, Parkinson disease.

COMMENT

The results of this study, to our knowledge the largest complete genomic screen in idiopathic PD, suggest that genetic factors are involved in the etiology of both early- and late-onset PD. These results are in contrast to findings of the twin study by Tanner and colleagues.[6] In that study of 161 twin pairs, the authors found similar concordance rates in identical (15.5%) and fraternal (11.1%) twin pairs and concluded that there was no role for genetics in late-onset PD. However, the authors acknowledged that there were limitations to the twin study design,[4] including sample size and time to follow-up.[25] Our results suggest that these concerns are warranted.

Analysis of 18 families with at least 1 early-onset PD case resulted in strong evidence for linkage to *D6S305*, which is located in intron 7 of the parkin gene. Parkin was originally described as the gene responsible for only autosomal recessive juvenile parkinsonism[8] and later was implicated in other autosomal recessive, early-onset PD.[9] The 18 families with early-onset PD were phenotypically similar to the overall data set, with the only notable difference being earlier age at onset of PD symptoms in at least 1 individual. The 18 families with early-onset PD did not have a recognizable mode of inheritance, contained a wide range of ages at onset (12-66 years), and were a mixture of affected sibpairs and other affected relative pairs in multiple generations.

Through examination of the parkin gene in these 174 families and an additional 134 multiplex and singleton families, we discovered parkin mutations in 11 early-onset and 7 late-onset idiopathic PD families.[26] These

linkage and mutation results indicate that parkin is an important genetic factor in PD and mutations are more prevalent than previously reported. Parkin mutations were not identified in 10 families with early-onset PD included in the genomic screen, indicating that additional loci underlying early-onset PD may exist.

In the 147 families with late-onset PD, the strongest overall evidence for linkage was on chromosome 17q at marker *D17S1293*, which is about 8 cM from the tau gene. The tau gene encodes a microtubule-associated protein that is expressed in the brain and forms paired helical filaments found in Alzheimer disease and other neurodegenerative disorders.[27] Mutations in the tau gene cause frontotemporal dementia with parkinsonism (FTDP),[28] and a haplotype of single nucleotide polymorphisms (SNPs) in the gene has been associated with progressive supranuclear palsy (PSP).[29]

The strict clinical criteria developed for use in this study ensured that these families have idiopathic PD and not atypical forms of parkinsonism. For example, to exclude potential cases of PSP from the sample, individuals with PD had to have asymmetrical motor symptoms at onset, no postural instability with falls early in the disease course, and no supranuclear down- or lateral-gaze palsy. Subjects with potential FTDP were excluded from the PD affected group by requiring the absence of dementia at onset and the presence of asymmetrical onset of motor symptoms. Cognitive status testing was not performed during the initial clinical examinations in these families, and therefore we cannot determine if linkage to this region is associated with development of dementia later in the disease. These data are being collected during follow-up evaluations of these families and will be examined in future studies. Therefore, the evidence for linkage of late-onset PD to chromosome 17q suggests a possible genetic link between FTDP, PSP, and idiopathic PD. We examined intragenic SNPs in tau for association with PD and found that a haplotype of 4 SNPs in the tau gene is significantly associated with increased risk of developing PD.[30] Association of PD with a haplotype of tau and evidence for linkage to that region of chromosome 17q suggest that tau or a gene in linkage disequilibrium with tau is a genetic risk factor for PD.

Stratification by levodopa response identified additional regions of interest. In particular, multipoint LODs on chromosome 9q were stronger in these 9 families compared with the other families with late-onset idiopathic PD. Each family included both levodopa-responsive and nonresponsive members, indicating that variable response to levodopa within the family might be part of the phenotype associated with a susceptibility locus in this region. This region of 9q contains the torsinA gene, deletions in which are responsible for causing idiopathic torsion dystonia, another movement disorder that is not responsive to levodopa therapy.[31] These data suggest a potential etiologic connection between dystonia and non–levodopa-responsive parkinsonism.

Family-based genetic linkage studies are frequently used as initial attempts to identify susceptibility genes in complex diseases. This study design requires a selected sample of families with multiple related individuals diagnosed as having the disease. Only 10% to 20% of individuals with PD report a family history of the disease, and only a subset have a living relative with PD. Because of this ascertainment scheme, these multiplex families may not be representative of all cases of PD. Therefore, the results of this study may not apply to all PD cases and cannot be used to calculate the relative contribution of each gene to the overall risk of PD. However, we found no significant differences in the clinical presentation of patients in this data set and those seen in a general PD clinic population.[24] Once susceptibility genes underlying each of these regions of linkage are identified, investigations of population-based random samples will be necessary to determine the contribution of each to the overall risk of idiopathic PD.

These results provide strong evidence that the parkin gene is influential in the development of early-onset PD, that several genes may influence the development of late-onset PD, and that age at onset and levodopa response pattern may be useful discriminators for genetic etiology. Like many complex traits, it is likely that PD is caused by an interaction of genetic and environmental risk factors, in which specific genetic templates are more susceptible to the influences of environmental exposures. Further studies to identify the molecular pathways affected by the responsible genes will provide valuable insight into this complex etiology and potential treatment for PD.

Author Affiliations: Department of Medicine and Center for Human Genetics, Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, NC (Drs W. K. Scott, B. L. Scott, Vance, and Pericak-Vance, Messrs Stajich, Slotterbeck, Booze, and Ribble, and Mss Rampersaud and West); Struthers Parkinson Center, Golden Valley, Minn (Dr Nance); Department of Neurology, Emory University School of Medicine, Atlanta, Ga (Dr Watts); Department of Neurology, Ohio State University, Columbus (Dr Hubble); Department of Neurology, University of Miami School of Medicine, Miami, Fla (Drs Koller and Lyons); Department of Neurology, University of Kansas Medical Center, Kansas City (Dr Pahwa); Department of Neurology, University of Pennsylvania Health System, Philadelphia (Drs Stern and Colcher); Department of Neurology, Marshfield Clinic, Marshfield, Wis (Dr Hiner); Department of Neurology, Baylor College of Medicine, Houston, Tex (Drs Jankovic and Ondo); Carolina Neurologic Clinic, Charlotte, NC (Dr Allen); Department of Neurological Sciences, Rush-Presbyterian-St Luke's Hospital, Chicago, Ill (Dr Goetz); Departments of Psychiatry and Behavioral Science and Neurology, University of California, Los Angeles (Drs Small and Masterman); Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Perth (Drs Mastaglia and Laing); GlaxoSmithKline Research and Development, Greenford, Middlesex, England (Drs Gibson and Middleton); GlaxoSmithKline Research and Development, Research Triangle Park, NC (Dr Roses); and Program in Human Genetics, Vanderbilt University Medical Center, Nashville, Tenn (Dr Haines).

Author Contributions: Dr Pericak-Vance had full access to all of the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: W. K. Scott, Nance, Hubble, Koller, Stern, Hiner, Jankovic, Goetz, Roses, Pericak-Vance.

Acquisition of data: W. K. Scott, Nance, Watts, Hubble, Koller, Lyons, Pahwa, Stern, Colcher, Hiner, Jankovic, Ondo, Allen, Goetz, Small, Masterman, Mastaglia, Laing, Stajich, Slotterbeck, Booze, Ribble, Rampersaud, West, Middleton, Roses, Haines, B. L. Scott, Vance.

Analysis and interpretation of data: W. K. Scott, Koller, Booze, Ribble.

Drafting of the manuscript: W. K. Scott, Koller, Booze, Ribble, Gibson, Haines.

Critical revision of the manuscript for important intellectual content: W. K. Scott, Nance, Watts, Hubble, Koller, Lyons, Pahwa, Stern, Colcher, Hiner, Jankovic, Ondo, Allen, Goetz, Small, Masterman, Mastaglia, Laing, Stajich, Slotterbeck, Booze, Rampersaud, West, Middleton, Roses, Haines, B. L. Scott, Vance, Pericak-Vance.
Statistical expertise: W. K. Scott, Haines.
Obtained funding: Roses, Pericak-Vance.
Administrative, technical, or material support: Watts, Hubble, Koller, Lyons, Pahwa, Stern, Hiner, Ondo, Small, Mastaglia, Laing, Slotterbeck, Booze, Ribble, Rampersaud, West, Middleton, Roses, Vance, Pericak-Vance.
Study supervision: W. K. Scott, Koller, Hiner, Booze, Pericak-Vance.
Clinical expertise: Goetz.

Funding/Support: This research was supported in part by National Institutes of Health grants P01 NS26630 (Dr Pericak-Vance) and P50 NS39764 (Dr Vance) and funding from GlaxoSmithKline Inc.
Acknowledgment: We thank all the families whose participation made this project possible. We gratefully acknowledge the contributions of the study personnel at each of the collaborating sites and at the Center for Human Genetics, Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, NC.

REFERENCES

1. Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. *Neurol Clin*. 1996;14:317-335.
2. Allan W. Inheritance of the shaking palsy. *Arch Intern Med*. 1937;60:424-436.
3. Sveinbjornsdottir S, Hicks A, Jonsson T, et al. Familial aggregation of Parkinson's disease in Iceland. *N Engl J Med*. 2000;343:1765-1770.
4. Johnson WG, Hodge SE, Duvoisin RC. Twin studies and the genetics of Parkinson's disease: a reappraisal. *Mov Disord*. 1990;5:187-194.
5. Vieregge P, Schiffke KA, Friedrich HJ, Müller B, Ludin HP. Parkinson's disease in twins. *Neurology*. 1992;42:1453-1461.
6. Tanner CM, Ottman R, Goldman SM, et al. Parkinson disease in twins: an etiologic study. *JAMA*. 1999; 281:341-346.
7. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science*. 1997;276: 2045-2047.
8. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*. 1998;392:605-608.
9. 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. *Hum Mol Genet*. 1999;8:567-574.
10. Gasser T, Müller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat Genet*. 1998;18:262-265.
11. Ward CD, Gibb WR. Research diagnostic criteria for Parkinson's disease. *Adv Neurol*. 1990;53:245-249.
12. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992;55:181-184.
13. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathological study. *Neurology*. 1992;42:1142-1146.
14. Vance JM, Ben Othmane K. Methods of genotyping. In: Haines JL, Pericak-Vance MA, eds. *Approaches to Gene Mapping in Complex Human Diseases*. New York, NY: Wiley-Liss; 1998:213-228.
15. Boehnke M, Cox NJ. Accurate inference of relationships in sib-pair linkage studies. *Am J Hum Genet*. 1997;61:423-429.
16. O'Connell JR, Weeks DE. The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. *Nat Genet*. 1995;11:402-408.
17. Ott J. *Analysis of Human Genetic Linkage*. 3rd ed. Baltimore, Md: The Johns Hopkins University Press; 1999.
18. Kong A, Cox NJ. Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet*. 1997; 61:1179-1188.
19. Risch N. Linkage strategies for genetically complex traits I: multilocus models. *Am J Hum Genet*. 1990; 46:222-228.
20. Golbe LI. Young-onset Parkinson's disease: a clinical review. *Neurology*. 1991;41:168-173.
21. Weeks DE, Lathrop GM. Polygenic disease: methods for mapping complex disease traits. *Trends Genet*. 1995;11:513-519.
22. Weeks DE, Conley YP, Mah TS, et al. A full genome scan for age-related maculopathy. *Hum Mol Genet*. 2000;9:1329-1349.
23. Pericak-Vance MA, Bass MP, Yamaoka LH, et al. Complete genomic screen in late-onset familial Alzheimer disease: evidence for a new locus on chromosome 12. *JAMA*. 1997;278:1237-1241.
24. Hubble JP, Weeks CC, Nance M, et al. Parkinson's disease: clinical features in sibships [abstract]. *Neurology*. 1999;52:A13.
25. Langston JW. Epidemiology versus genetics in Parkinson's disease: progress in resolving an age-old debate. *Ann Neurol*. 1998;44(suppl 1):S45-S52.
26. Scott WK, Rogala AR, Rampersaud E, et al. Parkin mutations and idiopathic Parkinson disease (PD) [abstract]. *Am J Hum Genet*. 2000;67(suppl):19.
27. Poorkaj P, Bird T, Wijsman E, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol*. 1998;43:815-825.
28. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998;393: 702-705.
29. Baker M, Litvan I, Houlden H, et al. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum Mol Genet*. 1999; 8:711-715.
30. Martin ER, Scott WK, Nance MA, et al. Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. *JAMA*. 2001;286: 2245-2250.
31. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet*. 1997;17:40-48.

Am. J. Hum. Genet. 71:124–135, 2002

Genome Screen to Identify Susceptibility Genes for Parkinson Disease in a Sample without *parkin* Mutations

Nathan Pankratz,[1] William C. Nichols,[2] Sean K. Uniacke,[2] Cheryl Halter,[1] Alice Rudolph,[3] Cliff Shults,[4,5] P. Michael Conneally,[1] Tatiana Foroud,[1] and the Parkinson Study Group

[1]Department of Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis; [2]Division of Human Genetics, Children's Hospital Medical Center, Cincinnati; [3]Department of Neurology, University of Rochester, Rochester, NY; and [4]Department of Neurosciences, University of California, and [5]Veterans Administration San Diego Healthcare System, San Diego

Parkinson disease (PD) is a common neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular rigidity, and postural instability, as well as by a clinically significant response to treatment with levodopa. Mutations in the *α-synuclein* gene have been found to result in autosomal dominant PD, and mutations in the *parkin* gene produce autosomal recessive juvenile-onset PD. We have studied 203 sibling pairs with PD who were evaluated by a rigorous neurological assessment based on (*a*) inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and (*b*) exclusion criteria highly associated with other, non-PD pathological diagnoses. Families with positive LOD scores for a marker in an intron of the *parkin* gene were prioritized for *parkin*-gene testing, and mutations in the *parkin* gene were identified in 22 families. To reduce genetic heterogeneity, these families were not included in subsequent genome-screen analysis. Thus, a total of 160 multiplex families without evidence of a *parkin* mutation were used in multipoint nonparametric linkage analysis to identify PD-susceptibility genes. Two models of PD affection status were considered: model I included only those individuals with a more stringent diagnosis of verified PD (96 sibling pairs from 90 families), whereas model II included all examined individuals as affected, regardless of their final diagnostic classification (170 sibling pairs from 160 families). Under model I, the highest LOD scores were observed on chromosome X (LOD score 2.1) and on chromosome 2 (LOD score 1.9). Analyses performed with all available sibling pairs (model II) found even greater evidence of linkage to chromosome X (LOD score 2.7) and to chromosome 2 (LOD score 2.5). Evidence of linkage was also found to chromosomes 4, 5, and 13 (LOD scores >1.5). Our findings are consistent with those of other linkage studies that have reported linkage to chromosomes 5 and X.

Introduction

Parkinson disease (PD [MIM 168600]) is a common neurodegenerative disorder affecting >1% of 55-year-old individuals and >3% of those >75 years of age (de Rijk et al. 1997). It is characterized by bradykinesia, resting tremor, muscular rigidity, and postural instability, as well as by a clinically significant response to treatment with levodopa (Gasser 2001). The pathology of PD involves the degeneration of brain dopaminergic pathways, mostly in the substantia nigra but also in other regions of the brain, and the presence of Lewy bodies in the substantia nigra (Gibb and Lees 1989; Fearnley and Lees 1991).

To better understand the role of genetics in PD, many investigators have initiated studies to estimate familial aggregation of PD in first- and/or second-degree relatives of patients with PD. Studies around the world have provided evidence that genetic risk factors are involved in the pathogenesis of the idiopathic form of PD. Estimates of the relative risk to first-degree relatives of an affected individual range from 2.7 (Kuopio et al. 2001) to 3.5 (Payami et al. 1994) in the United States and are 2.9 in Finland (Autere et al. 2000), 6.7 in Iceland (Sveinbjornsdottir et al. 2000), 7.7 in France (Preux et al. 2000), 3.2 in three centers within Europe (Elbaz et al. 1999), 5.0 in Canada (Uitti et al. 1997), 13.4 in Italy (De Michele et al. 1995), and 7.1 in Germany (Vieregge et al. 1994).

Several families with Mendelian segregation of PD have been reported. At present, four genes implicated in autosomal dominant forms of parkinsonism have been identified or localized. The alpha-synuclein gene was identified by studying a large Italian kindred in which PD was pathologically confirmed (Polymeropoulos et al. 1997). The same alpha-synuclein mutation, G209A, observed in the Italian kindred was later found in three Greek families, most of which can trace their ancestry to a very small geographical area on the Pelo-

Received March 11, 2002; accepted for publication April 15, 2002; electronically published June 7, 2002.

Address for correspondence and reprints: Dr. Tatiana Foroud, Department of Medical and Molecular Genetics (IB 130), 975 West Walnut Street, Indianapolis, IN 46202-5251. E-mail: tforoud@iupui.edu

0002-9297/2002/7101-0013$15.00

ponnisos in southern Greece (Polymeropoulos et al. 1997). Alpha-synuclein mutations in eight additional individuals from six different families from central and southwestern Greece have been reported more recently (Bostantjopoulou et al. 2001). In view of the close historical ties to southern Italy, these mutation results suggest the presence of a founder effect (Gasser 2001). Subsequently, another mutation, G88C, in the gene was identified in a German family with autosomal PD (Kruger et al. 1998). But, since no other mutations in alpha-synuclein have been identified in the large number of patients with sporadic or familial PD that have been screened (Chan et al. 1998; Farrer et al. 1998; Vaughan et al. 1998; Pastor et al. 2001), alpha-synuclein is most likely not a major risk factor in familial PD. A mutation, Ile93Met, in the ubiquitin carboxy-terminal hydrolase-L1 gene was identified in two individuals with PD who were members of a German pedigree (Leroy et al. 1998). The PARK3 locus on chromosome 2p13 has been linked in a subset of families with German ancestry (Gasser et al. 1998), although the responsible gene has not yet been identified. Last, the PARK4 locus on chromosome 4p14-16.3 has been linked in a four-generation pedigree (Farrer et al. 1999). Despite these promising findings, pedigrees with autosomal dominant PD are rare and seem to represent only a small number of families with PD (The French Parkinson's Disease Genetics Study Group 1998).

Autosomal recessive juvenile parkinsonism (ARJP [MIM 600116]) is a distinct clinical and genetic entity within familial PD. It is characterized by typical PD features and an early (<40 years) age at onset, slow progression of the disease, sustained response to levodopa, early levodopa-induced complications (fluctuations and dyskinesias) that are often severe, hyperreflexia, and mild dystonia, mainly in the feet (Yamamura et al. 1973; Ishikawa and Tsuji 1996). Among the genes implicated in familial PD, the largest number of mutations have been found in the *parkin* gene (Kitada et al. 1998), and mutations in this gene might account for PD in as many as 50% of familial patients with ARJP (Lücking et al. 2000). Two other loci have been implicated in autosomal recessive early-onset parkinsonism: PARK6 has been localized on chromosome 1p35-36 in a single Italian family (Valente et al. 2001), and PARK7 has been mapped to chromosome 1p37 in a consanguineous pedigree from a genetically isolated population in the southwestern region of the Netherlands (van Duijn et al. 2001).

There is clear evidence of a single Mendelian gene in a minority of families with PD. However, evidence of a genetic contribution to more typical, late-onset PD has not been universal. A report of a low concordance rate of PD in a sample of World War II veteran twins has been interpreted to imply that the role of genes in PD susceptibility is minimal. Further analyses of these data suggest greater twin concordance among twins with early-onset PD (Tanner et al. 1999). A major limitation of most twin studies is that they are usually cross-sectional in nature. In the case of PD, in which the age at onset is quite variable, a cross-sectional study may fail to identify concordant twin pairs with widely differing ages at onset. In one instance, the age at onset of PD in a pair of MZ twins differed by 20 years (Dickson et al. 2001). Functional imaging of the brain has suggested that some apparently normal cotwins actually have decreased function of the nigrostriatal dopaminergic system and may be presymptomatic, implying that the concordance rates for both MZ and DZ twins may be higher than previously estimated (Burn et al. 1992; Piccini et al. 1997).

The purpose of the present study was to identify genes contributing to PD susceptibility, particularly among families with more typical, later-onset PD. Since, to date, mutations in *parkin* are the most common inherited defect identified in PD, our initial efforts were focused on identifying those families likely to have *parkin* mutations, so that they could be further screened and eliminated from the genome-screen analyses. Our intention was to reduce the genetic heterogeneity in our sample and, thereby, to increase our power to detect non-*parkin* PD-susceptibility genes. Our study provides evidence of PD-susceptibility loci in several chromosomal regions.

Subjects and Methods

Subjects

Families consisting of at least one pair of living siblings diagnosed with PD were recruited through 60 Parkinson Study Group (PSG) sites located throughout North America. The sample was primarily white (94%), although Hispanics (6%) also participated. All study participants completed a uniform clinical evaluation that consisted of parts II and III of the Unified Parkinson Disease Rating Scale (Lang and Fahn 1989). A diagnostic checklist also was completed by a PSG movement-disorder specialist, with inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and with exclusion criteria highly associated with other, non-PD pathological diagnoses (see the Appendix) (Hughes et al. 1992*a*, 1992*b*). Responses on the diagnostic checklist were then used to classify study subjects as having verified PD (285 subjects) or nonverified PD (99 subjects). Peripheral blood was obtained from all individuals after appropriate written informed consent approved by each individual institution's institutional review board was completed. DNA was prepared by standard methods (Madisen et al. 1987).

Table 1

Demographics of Sample

Model	No. of Families	No. of Pairs	% Male	Mean ± SD Age at Onset (years)
I (183 subjects)	90	96	60	62.3 ± 9.7 (range 32–83)
II (325 subjects)	160	170	61	62.8 ± 10.7 (range 18–83)

One of the advantages of our study is the use of the diagnostic checklist for the classification of disease status. The inclusion criteria, consisting of clinical features highly associated with autopsy-confirmed PD, in conjunction with the exclusion criteria, consisting of clinical features highly associated with other, non-PD pathological diagnoses, provide the stringent diagnostic criteria essential for the successful identification of PD-susceptibility genes. The high interrater and intersite reliability of the diagnostic instrument provided further reassurance that error in diagnosis was kept at a minimum (Siemers et al. 1998).

parkin *Screening*

A marker (D6S305) in intron 7 of the *parkin* gene was genotyped in all study subjects. Families with positive LOD scores at this marker, under an autosomal recessive model of disease inheritance ($n = 68$), and families with an affected family member with an age at onset of <45 years ($n = 21$) were screened for *parkin* mutations, by both direct-sequencing and fluorescent-dosage analysis (Nichols et al., in press). Twenty different *parkin* mutations were identified in 22 of the 74 families analyzed. Genetic analyses were performed with and without the families with a *parkin* mutation.

Genotyping

A genome screen was completed by use of 400 dinucleotide markers, from the ABI Prism Linkage Mapping Set (Applied Biosystems), that had an average heterozygosity of 79% and an average intermarker spacing of 8.6 cM. The average information content for the 23 chromosomes was 0.65 when estimated every 1 cM and was 0.71 when estimated at a marker (Mapmaker/Sibs; Kruglyak and Lander 1995). In brief, 30 ng of genomic DNA was PCR amplified by use of each marker, in a 10-μl reaction. After PCR, the PCR products were pooled by use of equal amounts of each PCR. One microliter of this multiplexed mix was added to 10 μl of formamide containing the GENESCAN-400HD ROX size standard (Applied Biosystems). Genotypes were determined by an ABI 3700 DNA Analyzer (Applied Biosystems) and GENESCAN 3.5, GENOTYPER 3.6, and GENEMAPPER 1.1 software.

All genotypic data were evaluated for Mendelian inheritance of marker alleles, by the program Pedcheck (O'Connell and Weeks 1998). The marker genotypic data were used to verify the full-sibling relationships among the subjects, by the computer program RELATIVE (Goring and Ott 1997). Three half-sibling pairs were eliminated from further analyses, because the sharing of marker alleles identical by descent was significantly lower than that which would be expected for full siblings.

Statistical Analysis

A total of 160 families ($n = 325$ individuals) who had no evidence of a *parkin* mutation and who had two or more affected siblings were available for genetic analyses. Of the two models of affection considered for this sample, model I included only those individuals with a more stringent diagnosis of verified PD ($n = 96$ sibling pairs, from 90 families), whereas model II included all examined individuals as affected, regardless of their final diagnostic classification ($n = 170$ sibling pairs, from 160 families). The majority of families consisted of a single pair of affected siblings. Under model I, there were 87 families with a single affected sibling pair and 3 families with three affected siblings. Under the broader disease definition employed in model II, there were 155 families with two affected siblings and 5 families with three affected siblings. The average age at onset of PD did not differ significantly ($P = .47$) between the sample employed in the model I analyses and the sample of individuals included in the more inclusive model II definition. The characteristics of the study population are listed in table 1.

Multipoint nonparametric linkage analysis was performed for both models of affection status, by the maximum-likelihood method implemented in the computer program Mapmaker/SIBS (Kruglyak and Lander 1995). Analysis was performed both with dominance variance fixed at zero and with dominance variance free to vary. Analyses were performed by employing all possible sibling pairs from families of size greater than two.

For completeness, genomewide linkage analyses also were performed with the entire collected sample, including those families with a known *parkin* mutation ($n =$ 182 families). In addition, to identify any potential loci acting epistatically with the *parkin* gene, a genomewide linkage analysis also was performed in a sample limited to the 22 families with a known *parkin* mutation.

Results

Results of the genome screen of the families with *parkin* mutations were reviewed (n = 160 families), and chromosomal regions with LOD scores >1.5 were identified for further evaluation (figs. 1 and 2 and table 2). Information content, both at linked markers and in the linked intermarker regions, was comparable to that observed in the full genome. When only PD sibling pairs meeting the stricter PD diagnostic criteria were included in the analyses (model I), three chromosomal regions had LOD scores >1.5 (fig. 1). The highest LOD scores were observed on chromosome X (LOD score 2.1), near marker DXS1001 (fig. 3), and on chromosome 2 (LOD score 1.9), near marker D2S206 (fig. 4). Three markers in this 21-cM region on chromosome X (i.e., markers DXS1106, DXS8055, and DXS1001) yielded LOD scores >1.5. Subsequent analyses suggested that the majority of the evidence of linkage to this region is derived from 35 brother-brother pairs (LOD score 1.8). Analyses of the 17 sister-sister pairs produced a maximum LOD score of 0.9 in this region of Xq. Linkage analyses employing the mixed-sex sibships (n = 44 sib pairs) yielded a maximum LOD score of 0.3. In addition, a LOD score of 1.6 was found for chromosome 5, near marker D5S471.

Analyses were also performed with all available sibling pairs (model II). With this larger sample of sibling pairs, LOD scores >1.5 were observed for chromosomes 2, 4, 13, and X (table 2 and fig. 2). The maximum LOD score obtained for the entire genome screen was for chromosome X (LOD score 2.7), near marker DXS1106 (fig. 3). Three markers in this 23-cM region (i.e., markers DXS1106, DXS8055, and DXS1001) yielded LOD scores >1.5. Subsequent analyses suggest that the majority of the evidence of linkage to this region is derived from 66 brother-brother pairs (LOD score 1.9). Analyses of the 29 sister-sister pairs produced a maximum LOD score of 0.65 for this region of Xq. Linkage analyses employing the mixed-sex sibships (n = 75 sib pairs) yielded a maximum LOD score of 0.24.

The maximum LOD score for chromosome 2, when the broader disease definition (model II) was used, was 2.5. Similar to what was seen in the model I analyses, the maximum LOD score for chromosome 2 was near marker D2S206 (fig. 4). Three markers in this region of chromosome 2, spanning 24 cM, produced LOD scores $\geq$1.5 (i.e., D2S396, D2S206, and D2S338). Thus, the two chromosomes with the highest LOD scores under the more restrictive model I diagnosis also had the highest LOD scores under the broader disease definition.

Two additional chromosomal regions had LOD scores above our initial threshold of 1.5. A maximum LOD score of 1.6 was found for chromosome 4, near marker D4S1597, and a maximum LOD score of 1.5 was found for chromosome 13, near marker D13S171.

Linkage analyses also were performed in the entire collected sample (n = 182 families), including the 22 families with an identified *parkin* mutation (table 2). For chromosomes 4, 5, and X, both the LOD score under both model I and that under model II were lower than those for the analyses performed without families with *parkin* mutations. For chromosome 13, the complete sample had a higher LOD score under model II only (LOD score 2.1 vs. LOD score 1.5). Analyses performed with the entire sample did identify one additional chromosomal region, on chromosome 10 (LOD score 1.6), that met our initial linkage threshold. Interestingly, for chromosome 2, the entire sample produced a substantially higher LOD score under model I (LOD score 3.1 vs. LOD score 1.9). To further examine the potential role of a PD-susceptibility locus on chromosome 2, as well as potential epistatic interactions between *parkin* and other loci, a genome screen was performed with only the 22 families with *parkin* mutations (fig. 5). In this small subset of the data, there was no evidence of a PD-susceptibility gene on chromosome 2; however, there was

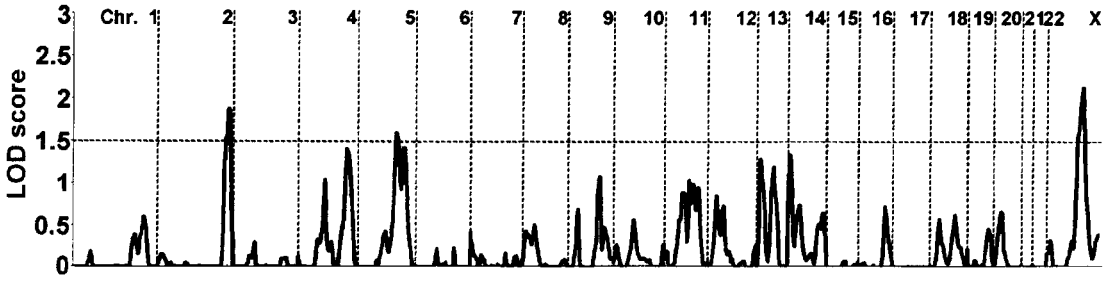


Figure 1 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, under the narrower, model I definition of PD diagnosis.

some evidence of linkage to chromosomes 8 (LOD score 1.6), 12 (LOD score 2.0), and 19 (LOD score 1.7).

Discussion

Three other studies are currently ongoing that seek to identify PD-susceptibility genes. The GenePD study, analyzing data from 113 sibling pairs, has reported LOD scores of 0.9–1.3 for chromosomes 1, 9, 10, and 15 (DeStefano et al. 2001). A second study, analyzing 174 extended pedigrees, including 378 affected individuals, has reported LOD scores of 1.5–2.5 for chromosomes 5, 8, 9, 14, 17, and X in families with late-onset PD (Scott et al. 2001). The deCODE study in Iceland has reported a LOD score of 4.9 on chromosome 1, as well as evidence of linkage to chromosomes 5, 7, 13, 14, and X (Hicks et al. 2001).

We have completed a genome screen in a large sample of 170 sibling pairs with PD that were ascertained from 160 families. Unlike previous PD-linkage studies, ours has attempted to reduce genetic heterogeneity by screening all families by use of a marker in the *parkin* gene, to identify families likely to have *parkin* mutations. This has allowed us to identify 22 families with *parkin* mutations who were then removed from subsequent genome-screen analyses. Linkage analyses performed with and without the families with *parkin* mutations suggest that most of the LOD scores that we have reported increased with the removal of the families with known *parkin* mutations (table 2).

In this study, the strongest evidence of linkage was reported for chromosome X. Evidence of linkage was observed both under the more restrictive (model I) and under the broader (model II) disease definitions. The maximum LOD score in each analysis was at a different marker; however, these markers are only 13 cM apart and likely represent the same underlying susceptibility gene. It is interesting that, in our sample, most of the evidence of linkage to this region on chromosome X came from the brother-brother pairs. It is intriguing to speculate that this linkage to chromosome X might explain the slightly higher incidence of PD among males (Tanner et al. 1992).

Table 2

Regions with LOD Scores ≥1.5

Chromosome	Position[a] (cM)	LOD Score: With Families with *parkin* Mutations, Model I	With Families with *parkin* Mutations, Model II	Without Families with *parkin* Mutations, Model I	Without Families with *parkin* Mutations, Model II
2	236	3.1	2.5	1.9	2.5
4	160	1.1	1.4	1.4	1.6
5	128	1.3	.7	1.6	.5
10	68	.6	1.6	.6	1.3
13	13-22	1.2	2.1	1.3	1.5
X	105-122	1.4	2.5	2.1	2.7

[a] Based on the sex-averaged genetic maps from the Center for Medical Genetics, Marshfield Medical Research Foundation.

Two other PD studies appear to report linkage to this same region of Xq21-25 (Hicks et al. 2001; Scott et al. 2001). Unfortunately, few details were provided, in either study, regarding the markers near the maximum LOD score. Also, neither study reported whether the evidence of linkage was limited to a sample subset consisting of brother pairs, as is the case in our study. Thus, it is difficult to verify whether the results from these other studies might represent converging lines of evidence supporting the presence of a PD-susceptibility gene on chromosome X.

Interestingly, X-linked dystonia parkinsonism (XDP), which has been reported at high incidence in Panay, The Philippines, has been linked to Xq13.1 (Lee et al. 2001). It is an adult-onset, highly penetrant, X-linked disorder that primarily afflicts men (male:female ratio 123:1). It is a severe, progressive disorder with onset typically occurring, with dystonic movements, during the 3rd or 4th decade. Approximately a decade after disease onset, the dystonia typically coexists or is replaced by parkinsonism. Neuropathology reveals pronounced atrophy of the

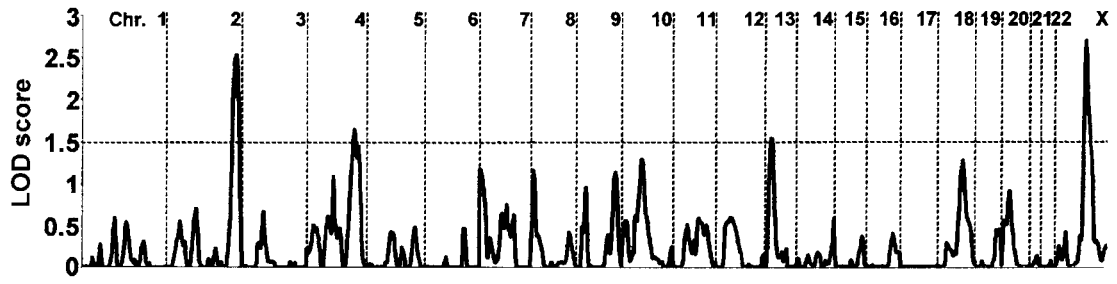


Figure 2 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, under the broader, model II definition of PD diagnosis.

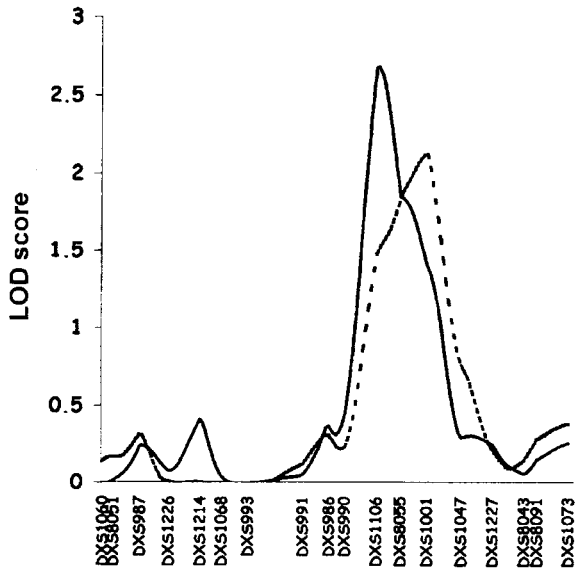


Figure 3 Multipoint LOD-score graph of chromosome X, under models I (*dashed line*) and II (*solid line*).

caudate and putamen, mostly in the cases with long-standing illness (Lee et al. 2001). The gene has been mapped to an <350-kb region in the DXS7117–DXS559 region (Nemeth et al. 1999), <20 cM from the region identified in our study. Our linkage finding may seem to be too far from the XDP linkage to represent the same gene. However, when, in our entire sample (n = 182 families), we review the data for chromosome 6, we find that the maximum LOD score (LOD score 1.1) occurs ~25 cM proximal to the actual location of the *parkin* gene. This is the case even when a marker is genotyped in exon 7 of the *parkin* gene. Thus, we believe that the *XDP* gene remains a possible positional candidate gene.

We have used complementary methods of analysis to ensure maximum power to detect loci contributing to PD susceptibility. Holmans (1993) has shown that analysis of affected-sib-pair data under the assumption of dominance variance, when Holmans's "possible triangle" is applied, appears to allow for a more sensitive test for putative genes acting in a recessive fashion than does analysis when dominance variance is fixed at zero. Several studies have reported higher estimates of relative risk for PD among siblings of affected individuals, compared with the risk of PD among the parents or offspring of affected individuals (Autere et al. 2000; Sveinbjornsdottir et al. 2000), suggesting evidence of at least some recessively acting PD-susceptibility genes. This would appear to be supported both by the recessively acting genes already implicated, such as *parkin*, and by the recent linkage findings on chromosome 1 (Vallente et al. 2001; van Duijn et al. 2001). We also have observed empirical data suggesting that LOD scores at a recessively acting locus increase when dominance variance is free to vary. Before the families with *parkin* mutations were removed, the LOD score for the *parkin* locus in the entire sample increased from 0.6 to 1.1 when dominance variance was free to vary; likewise, when the sample was limited to only the 22 families with *parkin* mutations, the LOD score increased from 5.0 to 7.6 (fig. 5).

In our study, the linkage findings for chromosome 2q are consistent with a recessively acting susceptibility locus. Allowing the dominance variance to vary increased the LOD score from 1.2 to 1.9 under model I and from 2.1 to 2.5 under model II. The linkage finding in this region was supported by three markers, all with LOD scores >1.5. None of the other three genomewide linkage studies (DeStefano et al. 2001; Hicks et al. 2001; Scott et al. 2001) have reported any evidence of linkage to chromosome 2.

The linkage to chromosome 4q32 was supported by four markers with a LOD score >1.0. In addition, another region, ~70 cM away, at 4q21, had a maximum LOD score of 1.1. None of the other three studies have reported linkage to either region (DeStefano et al. 2001; Hicks et al. 2001; Scott et al. 2001). The strongest candidate gene in this region is alpha-synuclein, which is near marker D4S1534 in the 4q21 region. Although only a few families with mutations in the alpha-synuclein gene have been identified (Chan et al. 1998; Farrer et al. 1998; Vaughan et al. 1998; Pastor et al. 2001), several recent studies have reported an association between PD and haplotypes in the promoter region of the alpha-synuclein gene (Kruger et al. 1999; Farrer et al. 2001*b*).

The evidence of linkage to chromosome 5q23 is supported by four markers with a LOD score >1.0. Similar to the linkage finding on chromosome 2, the results of analyses performed on chromosome 5, under model I, suggest that this locus also may be a recessively acting susceptibility locus; the maximum LOD score increased from 0.9 to 1.6 when linkage analyses were performed with dominance variance allowed to vary. Unlike the other linkage findings reported in this study, for this region there is a large disparity between the maximum LOD score under model I and that under model II. The higher LOD scores under model I may provide insight with regard to the putative gene's mode of action. "Parkinsonism" is a term referring to all clinical states characterized by tremor, muscle rigidity, and slowed movement (termed "bradykinesia") (Koller and Hubble 1992) and includes secondary forms of parkinsonism, such as those resulting from drug exposure and stroke. PD is the most common form of parkinsonism. We anticipate that model II includes some subjects with parkinsonism but

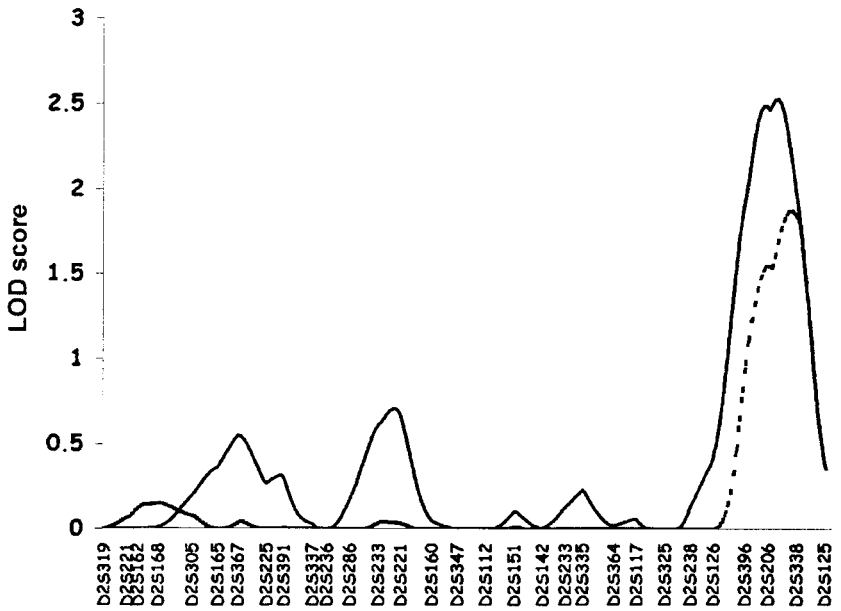


Figure 4 Multipoint-LOD-score graph of chromosome 2, under model I (*dashed line*) and II (*solid line*)

without PD. Perhaps the 5q23 locus is a PD-specific susceptibility locus, whereas most of the other loci mapped in this study may confer more-general parkinsonism susceptibility. Alternatively, the same genes may be contributing to both model I and model II; however, analyses performed with the broader disease model (i.e., model II) may result in higher LOD scores simply because the sample size is greater, as is the power to detect genetic effects.

Two other studies have reported linkage to 5q23 (Hicks et al. 2001; Scott et al. 2001). Scott et al. reported a multipoint LOD score of 1.5 for marker D5S816, which is <10 cM from marker D5S471, where the maximum LOD score was found. Within this cytogenetic band is a gene that is an excellent potential candidate, the synphilin-1 gene (*SNCAIP*). Normal parkin ubiquitinates synphilin-1 as well as the glycosylated form of alpha-synuclein. It is proposed that, without proper ubiquitination, these molecules do not properly degrade and that the accumulation of these proteins thus becomes toxic. Notably, all three of these proteins are present in the intracytoplasmic inclusions called "Lewy bodies" (Chung et al. 2001). No mutations in *SNCAIP* have been discovered in a small sample of patients with PD who have been screened in other studies (Bandopadhyay et al. 2001; Farrer et al. 2001*a*).

Linkage near the centromere of chromosome 13 is supported by two markers with LOD scores >1.0. Hicks et al. (2001) have reported linkage near this region, although the exact position and magnitude of their linkage finding has not been reported. One attractive candidate gene in this region is the copper-transporting P-type ATPase gene (*ATP7B*), which, if mutated, results in the autosomal recessive disorder Wilson disease. Cox et al. (1972) referred to the two "typical" forms of Wilson disease as being the "Slavic" type and the "juvenile" type, with the latter being a differential diagnosis considered for patients presenting with early-onset parkinsonism. The Slavic type has a late age at onset and is predominantly a neurologic disease that, like PD, affects the basal ganglia and can manifest tremor, dysarthria, poor motor coordination, and dementia. Approximately 1% of the general population are thought to be carriers of Wilson disease (Riordan and Williams 2001). These individuals typically do not have any clinical sequelae; however, they do have problems with copper metabolism. This is significant, because copper concentration in the cerebrospinal fluid has been reported at significantly increased levels in patients with idiopathic PD, compared with that in control subjects (Pall et al. 1987). Furthermore, alpha-synuclein has been shown to undergo self-oligomerization in the presence of copper(II) (Paik et al. 2000; Kim et al. 2001; Uversky et al. 2001), which may lead to the protein aggregation and neurodegeneration found in both PD and Alzheimer disease.

Similar to studies of other complex genetic disorders, previous genetic studies of PD have reported several linkage findings that were not replicated in our study. Most notable is the linkage to chromosome 1, reported by Hicks et al. (2001), in a sample of 117 affected individ-

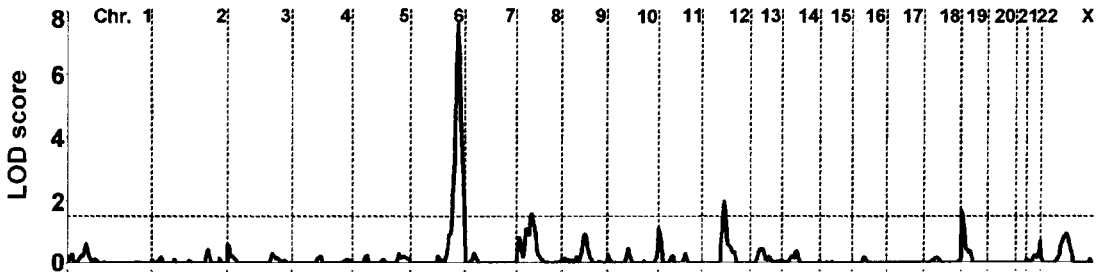


Figure 5 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, for the 22 families with *parkin* mutations.

uals from the genetically isolated population of Iceland. In that study, the result for chromosome 1 was the most significant linkage result, with a LOD score of 4.9. However, we found no evidence of linkage to this region (LOD score <0.6, both under model I and under model II). Importantly, no evidence of linkage near the centromere of chromosome 1 was reported in the two other genomewide studies of PD. It is therefore possible that a PD-susceptibility gene on chromosome 1 is present at high frequency only in the Icelandic population.

The study by Scott et al. (2001) found the most significant linkage on chromosome 17, near the *tau* gene. The strength of this linkage finding was increased when analyses were limited to a subset of families in which at least one individual in the kindred was not responsive to levodopa treatment. Since a positive response to this dopamine precursor is very common among individuals with PD, Scott et al. considered this potential phenotypic heterogeneity to be indicative of genotypic heterogeneity. The most notable improvements to LOD scores were for chromosomes 3, 9, and 17. Exploratory analyses were performed in our sample of only nine families, containing 11 sibling pairs, in which at least one member of the family was not responsive to levodopa. In this very small data set as well, as in analyses performed with either model I or model II, we found no evidence of linkage to *tau* (LOD scores 0.2, 0.0, and 0.0, respectively). Unlike our study of a sample primarily comprising sibling pairs, the study by Scott et al. (2001) included many extended pedigrees. Thus, it is possible that some of the families in that study are segregating a mutation in *tau*, which might produce an autosomal dominant pattern of PD inheritance.

In our sample of 170 affected sibling pairs, we have identified two chromosomal regions with particularly strong evidence of linkage. The findings for chromosomes 2 and X suggest the presence of loci that contribute to PD susceptibility, both in our analysis employing a narrow disease definition and in our analysis employing a more broader disease classification. These findings may suggest loci contributing to parkinsonism susceptibility—rather than simply to PD susceptibility per se. Importantly, our study has provided additional evidence of linkage, in an independent sample, to chromosomes 5 and X. We are continuing to recruit families with multiple living members diagnosed with PD, to replicate the results found for chromosomal regions identified in these analyses. An important advantage of our study is the identification and removal, prior to the genome-screen analyses, of families with *parkin* mutations. This has reduced genetic heterogeneity in our sample and, in most instances, has increased the LOD score for linked chromosomal regions.

Acknowledgments

This project was supported by National Institutes of Health grant R01 NS37167. We thank two anonymous reviewers for their helpful suggestions. We thank the subjects for their participation in this research study. PSG Investigators are as follows: *Steering Committee*—Lawrence Golbe, M.D. (UMDNJ Robert Wood Johnson Medical Center, New Brunswick, NJ); William Koller, M.D. (University of Miami, Miami); Karen Marder, M.D. (Columbia-Presbyterian Medical Center, New York); Frederick Marshall, M.D., David Oakes, Ph.D., Alice Rudolph, Ph.D., and Aileen Shinaman, J.D. (University of Rochester, Rochester, NY); and Eric Siemers (Eli Lilly & Company, Indianapolis). *Participating Investigators and Coordinators*—Julie Carter, R.N., M.D., A.N.P., Richard Camicioli, M.D., and Pamela Andrews, R.N. (Oregon Health & Science University, Portland); Joanne Wojcieszek, M.D., and Joann Belden, R.N. (Indiana University School of Medicine, Indianapolis); Magali Frenandez, M.D., Jean Hubble, M.D., and Carson Reider, Ph.D. (Ohio State University, Columbus); Ali Rajput, M.D., Alex Rajput, M.D., and Theresa Shirley, R.N. (Saskatoon Health District Board, Saskatoon, Saskatchewan, Canada); Michael Panisset, M.D., and Jean Hall, R.N. (McGill Centre for Studies in Aging, Verdun, Quebec, Canada); Tilak Mendis, M.D., David A. Grimes, M.D., and Peggy Gray, R.N., B.S.C.N. (Ottawa Civic Hospital, Ottawa, Ontario, Canada); Carmen Serrano Ramos, M.D., and Sandra Roque, R.N. (University of Puerto Rico School of Medicine, San Juan); Stephen Reich, M.D., and Becky

Dunlop, R.N. (Johns Hopkins University, Baltimore); Robert Hauser, M.D., Juan Sanchez-Ramos, M.D., Theresa Zesiewicz, M.D., and Holly Delgado, R.N. (University of South Florida, Tampa); Ronald Pfeiffer, M.D., and Brenda Pfeiffer, R.N., B.S.N. (University of Tennessee-Memphis, Memphis); Joseph Friedman, M.D., Hubert Fernandez, M.D., and Margaret Lannon, R.N., M.S. (Brown University, Pawtucket, RI); Cliff Shults, M.D., and Deborah Fontaine, R.N.C., G.N.P. (University of California–San Diego, San Diego); Lauren Seeberger, M.D., Christopher O'Brien, M.D., and Deborah Judd, R.N. (Colorado Neurological Institute, Englewood); Rajesh Pahwa, M.D., and Stephanie Thomas, L.P.N. (Kansas University Medical Center, Kansas City); Lawrence Elmer, M.D., Ph.D., and Kathy Davis, R.N., M.S.N. (Medical College of Ohio, Toledo); Danna Jennings, M.D., Kenneth Marek, M.D., and Susan Mendick, M.P.H. (Institute for Neurodegenerative Disorders, New Haven, CT); Daniel Truong, M.D., Mayank Pathak, M.D., and Anhoa Tran, R.N. (Parkinson's & Movement Disorder Institute, Fountain Valley, CA); Robert Rodnitzyk, M.D., and Judith Dobson, R.N. (University of Iowa, Iowa City); Roger Kurlan, M.D., and Debra Berry, M.S.N., N.P. (University of Rochester Medical Center, Rochester, NY); Paul Tuite, M.D., and Robyn Schacher, R.N. (University of Minnesota/Hennepin County Medical Center, Minneapolis); Michael Aminoff, M.D., F.R.C.P., and Mariann DiMinno (University of California San Francisco, San Francisco); Karen Marder, M.D., and Juliette Harris, M.S., Ph.D. (Columbia-Presbyterian Medical Center, New York); Peter Lewitt, M.D., and Maryan DeAngelis, R.N. (Clinical Neuroscience Center, West Bloomfield, MI); William Koller, M.D., William Weiner, R.N., and Kelly Lyons, Ph.D. (University of Miami, Miami); Wayne Martin, M.D., and Marguerite Wieler, B.S.C., P.T. (University of Alberta, Edmonton, Alberta, Canada); Joseph Jankovic, M.D., and Christine Hunter, R.N. (Baylor College of Medicine, Houston); John Bertoni, M.D., Ph.D., and Carolyn Peterson, R.N. (Creighton University, Omaha); Stewart Factor, D.O., and Sharon Evans, L.P.N. (Albany Medical College, Albany, NY); Francis Walker, M.D., and Victoria Hunt, R.N. (Wake Forest University School of Medicine, Winston-Salem, NC); Un Jung Kang, M.D., and Shirley Uy (University of Chicago, Chicago); Mark Stacy, M.D., and Kelli Williamson, R.N., (Barrow Neurological Institute, Phoenix); David Simon, M.D., and Lisa Scollins, R.N. (Beth Israel Deaconess Medical Center, Boston); Karen Brindauer, M.D., and Jeannine Petit, C.N.R.N., G.N.P.C. (Medical College of Wisconsin, Milwaukee); Bala Manyam, M.D., and Patricia Simpson, R.N., B.S.N. (Scott & White Hospital, Temple, TX); Anette Nieves, M.D., and Julie So (Toronto Western Hospital, Toronto, Ontario, Canada); Miodrag Velickovic, M.D., and Sabrina Phipps (Mount Sinai Medical Center, New York); Mark F. Gordon, M.D., and Joanna Hamann (Long Island Jewish Medical Center, New Hyde Park, NY); Maureen Leehey, M.D., and Sharon Culver, A.N.P. (University of Colorado Health Sciences Center, Denver); Paul Gordon, M.D., and Joan Werner (University of New Mexico, Albuquerque); Jayaraman Rao, M.D., and Maureen Cook, R.N., B.S.N. (LSU Medical Center, New Orleans); Arif Dalvi, M.D., and Donna Schweiterman (University of Cincinnati Medical Center, Cincinnati); Brad Racette, M.D. (Washington University, St. Louis); Kapil Sethi, M.D., and Joan Carpenter, R.N. (Medical College of Georgia, Augusta); Lewis Sudarsky, M.D., and Claire Corwin, P.A.C., B.S. (Brigham & Women's Hospital, Boston); Rachel Saunders Pullman, M.D., and Karyn Boyer (Beth Israel Medical Center, New York); Tanya Simuni, M.D., and Michele Wolff (Northwestern University Medical School, Chicago); Richard Dewey, M.D., and Melinda Jones, R.N., B.S.N. (University of Texas Southwestern Medical Center, Dallas); Neal Hermanowicz, M.D. (University of California Irvine, Irvine); Andrew Feigin, M.D., and Barbara Shannon, R.N. (North Shore University Hospital, Manhasset, NY); Vincent Calabresse, M.D., and Peggy Roberge, R.N. (McGuire Research Institute, Richmond); James Sutton, M.D., and Brad Hutchinson, C.C.R.C. (California Medical Clinic for Movement Disorders, Oxnard); Todd Ajax, M.D., and Janet Mannetter, R.N. (McFarland Clinic, Mary Greely Hospital, Ames, IA); G. David Podakalny, M.D., and Lisa Giffin, L.P.N. (New Jersey School of Osteopathic Medicine, Stratford); Oksana Suchowersky, M.D., and Mary Lou Klimek, R.N., B.N., M.A. (University of Calgary, Calgary, Alberta, Canada); Ryan Uitti, M.D., and Margaret Foster Turk, R.N. (Mayo Clinic Jacksonville, Jacksonville); Lisa Shulman, M.D., and Kelly Dustin, R.N. (University of Maryland School of Medicine, Baltimore); and *Biostatistics and Clinical Trials Coordination Center Staff*—David Oakes Ph.D., Arthur Watts, B.S., Antai Wang, M.A., Tori Ross, M.A., Susan Bennett, A.A.S., and Elaine Julian-Baros (University of Rochester, Rochester, NY).

Appendix

Inclusion and Exclusion Criteria

Inclusion Criteria

Age at onset >20 years
Bradykinesia
Clinician's overall impression that the subject has PD is >50%
At least one of the following:
  Muscular rigidity
  Rest tremor
  Postural instability
At least two of the following:

Persistent asymmetry of signs
Progressive disorder
Rest tremor
>50% Levodopa response
Levodopa-induced chorea
Levadopa response for ⩾5 years
Clinical course of ⩾10 years

Exclusion Criteria

Unexplained upper motor-neuron signs
History of repeated strokes with stepwise progression of parkinsonian features
History of encephalitis
History of oculogyric crisis
Clinical features/course suggesting Alzheimer disease or other dementia distinct from PD
Cortical sensory deficits or apraxia
Parkinsonism likely due to antidopaminergic drug use
Sustained remission of parkinsonian symptoms
Strictly unilateral features after 3 years
Supranuclear gaze palsy with down gaze <50% of normal
Cerebellar signs
Symptomatic orthostatic hypotension early in the course of the disease
Failure to respond to large dosage of levodopa
Brain imaging (by computed tomography or magnetic-resonance imaging) reveals presence of structural lesion(s) likely causing or contributing to parkinsonism

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PD [MIM 168600] and ARJP [MIM 600116])

References

Autere JM, Moilanen JS, Myllyla VV, Majamaa K (2000) Familial aggregation of Parkinson's disease in a Finnish population. J Neurol Neurosurg Psychiatry 69:107–109

Bandopadhyay R, de Silva R, Khan N, Graham E, Vaughan J, Engelender S, Ross C, Morris H, Morris C, Wood NW, Daniel S, Lees A (2001) No pathogenic mutations in the synphilin-1 gene in Parkinson's disease. Neurosci Lett 307: 125–127

Bostantjopoulou S, Katsarou Z, Papadimitriou A, Veletza V, Hatzigeorgiou G, Lees A (2001) Clinical features of parkinsonian patients with the alpha-synuclein (G209A) mutation. Mov Disord 16:1007–1013

Burn DJ, Mark MH, Playford ED, Maraganore DM, Zimmerman TR Jr, Duvoisin RC, Harding AE, Marsden CD, Brooks DJ (1992) Parkinson's disease in twins studied with 18F-dopa and positron emission tomography. Neurology 42:1894–1900

Chan P, Jiang X, Forno LS, Di Monte DA, Tanner CM, Langston JW (1998) Absence of mutations in the coding region of the alpha-synuclein gene in pathologically proven Parkinson's disease. Neurology 50:1136–1137

Chung KK, Zhang Y, Lim KL, Tanaka Y, Huang H, Gao J, Ross CA, Dawson VL, Dawson TM (2001) Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. Nat Med 7:1144–1150

Cox DW, Fraser FC, Sass-Kortsak A (1972) A genetic study of Wilson's disease: evidence for heterogeneity. Am J Hum Genet 24:646–666

De Michele G, Filla A, Marconi R, Volpe G, D'Alessio A, Scala R, Ambrosio G, Campanella G (1995) A genetic study of Parkinson's disease. J Neural Transm Suppl 45:21–25

de Rijk MC, Tzourio C, Breteler MM, Dartigues JF, Amaducci L, Lopez-Pousa S, Manubens-Bertran JM, Alperovitch A, Rocca WA (1997) Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study: European Community Concerted Action on the Epidemiology of Parkinson's disease. J Neurol Neurosurg Psychiatry 62 :10–15

DeStefano AL, Golbe LI, Mark MH, Lazzarini AM, Maher NE, Saint-Hilaire M, Feldman RG, et al (2001) Genome-wide scan for Parkinson's disease: the GenePD Study. Neurology 57:1124–1126

Dickson D, Farrer M, Lincoln S, Mason RP, Zimmerman TR Jr, Golbe LI, Hardy J (2001) Pathology of PD in monozygotic twins with a 20-year discordance interval. Neurology 56:981–982

Elbaz A, Grigoletto F, Baldereschi M, Breteler MM, Manubens-Bertran JM, Lopez-Pousa S, Dartigues JF, Alperovitch A, Tzourio C, Rocca WA (1999) Familial aggregation of Parkinson's disease: a population-based case-control study in Europe: EUROPARKINSON Study Group. Neurology 52:1876–1882

Farrer M, Destee A, Levecque C, Singleton A, Engelender S, Becquet E, Mouroux V, Richard F, Defebvre L, Crook R, Hernandez D, Ross CA, Hardy J, Amouyel P, Chartier-Harlin MC (2001*a*) Genetic analysis of synphilin-1 in familial Parkinson's disease. Neurobiol Dis 8:317–323

Farrer MJ, Gwinn-Hardy K, Muenter M, DeVrieze FW, Crook R, Perez-Tur J, Lincoln S, Maraganore D, Adler C, Newman S, MacElwee K, McCarthy P, Miller C, Waters C, Hardy J (1999) A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor. Hum Mol Genet 8: 81–85

Farrer M, Maraganore DM, Lockhart P, Singleton A, Lesnick TG, de Andrade M, West A, de Silva R, Hardy J, Hernandez D (2001*b*) alpha-Synuclein gene haplotypes are associated with Parkinson's disease. Hum Mol Genet 10:1847–1851

Farrer M, Wavrant-De Vrieze F, Crook R, Boles L, Perez-Tur J, Hardy J, Johnson WG, Steele J, Maraganore D, Gwinn K, Lynch T (1998) Low frequency of alpha-synuclein mutations in familial Parkinson's disease. Ann Neurol 43:394–397

Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114(pt 5): 2283–2301

French Parkinson's Disease Genetics Study Group, The (1998) alpha-Synuclein gene and Parkinson's disease. Science 279: 1116–1117

Gasser T (2001) Genetics of Parkinson's disease. J Neurol 248: 833–840

Gasser T, Müller-Myhsok B, Wszolek ZK, Oehlmann R, Calne DB, Bonifati V, Bereznai B, Fabrizio E, Vieregge P, Horstmann RD (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet 18:262–265

Gibb WR, Lees AJ (1989) The significance of the Lewy body in the diagnosis of idiopathic Parkinson's disease. Neuropathol Appl Neurobiol 15:27–44

Goring HH, Ott J (1997) Relationship estimation in affected sib pair analysis of late-onset diseases. Eur J Hum Genet 5: 69–77

Hicks A, Petursson H, Jónsson T, Stefánsson H, Jóhannsdóttir H, Sainz J, Frigge ML, Kong A, Gulcher JR, Stefánsson K and Sveinbjörnsdóttir S (2001) A susceptibility gene for late-onset idiopathic Parkinson's disease successfully mapped. Am J Hum Genet Suppl 69:200

Holmans P (1993) Asymptotic properties of affected-sib-pair linkage analysis. Am J Hum Genet 52:362–374

Hughes AJ, Ben-Schlomo Y, Daniel SE, Lees AJ (1992*a*) What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. Neurology 42: 1142–1146

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992*b*) Accuracy of the clinical diagnosis of Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55:181–184

Ishikawa A, Tsuji S (1996) Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile Parkinsonism. Neurology 47:160–166

Kim YS, Lee D, Lee EK, Sung JY, Chung KC, Kim J, Paik SR (2001) Multiple ligand interaction of α-synuclein produced various forms of protein aggregates in the presence of Aβ25-35, copper, and eosin. Brain Res 908:93–98

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. Nature 392:605–608

Koller WC, Hubble JP (1992) Classification of parkinsonism. In: Koller WC (ed) Handbook of Parkinson's disease. Marcel Dekker, New York, pp 59–103

Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106–108

Kruger R, Vieira-Saecker AM, Kuhn W, Berg D, Muller T, Kuhnl N, Fuchs GA, Storch A, Hungs M, Woitalla D, Przuntek H, Epplen JT, Schols L, Riess O (1999) Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. Ann Neurol 45:611–617

Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454

Kuopio A, Marttila RJ, Helenius H, Rinne UK (2001) Familial occurrence of Parkinson's disease in a community-based case-control study. Parkinsonism Relat Disord 7:297–303

Lang AE, Fahn S (1989) Assessment of Parkinson's disease. In: Munsat TL (ed) Quantification of neurologic deficit. Butterworth, Boston, pp 285–309

Lee LV, Munoz EL, Tan KT, Reyes MT (2001) Sex linked recessive dystonia parkinsonism of Panay, Philippines (XDP). Mol Pathol 54:362–368

Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH (1998) The ubiquitin pathway in Parkinson's disease. Nature 395:451–452

Lücking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denefle P, Wood NW, Agid Y, Brice A (2000) Association between early-onset Parkinson's disease and mutations in the Parkin gene: French Parkinson's Disease Genetics Study Group. N Engl J Med 342: 1560–1567

Madisen L, Hoar DI, Holroyd CD, Crisp M, Hodes ME (1987) DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. Am J Med Genet 27:379–390

Nemeth AH, Nolte D, Dunne E, Niemann S, Kostrzewa M, Peters U, Fraser E, Bochukova E, Butler R, Brown J, Cox RD, Levy ER, Ropers HH, Monaco AP, Muller U (1999) Refined linkage disequilibrium and physical mapping of the gene locus for X-linked dystonia-parkinsonism (DYT3). Genomics 60:320–329

Nichols WC, Pankratz N, Uniacke SK, Pauciulo MW, Halter

C, Rudolph A, Conneally PM, Foroud T, the Parkinson Study Group. Linkage stratification and mutation analysis at the parkin locus identifies mutation positive, Parkinson disease families. Hum Genet (in press)

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266

Paik SR, Shin HJ, Lee JH (2000) Metal-catalyzed oxidation of alpha-synuclein in the presence of copper(II) and hydrogen peroxide. Arch Biochem Biophys 378:269–277

Pall HS, Williams AC, Blake DR, Lunec J, Gutteridge JM, Hall M, Taylor A (1987) Raised cerebrospinal-fluid copper concentration in Parkinson's disease. Lancet 2:238–241

Pastor P, Munoz E, Ezquerra M, Obach V, Marti MJ, Valldeoriola F, Tolosa E, Oliva R (2001) Analysis of the coding and the 5′ flanking regions of the alpha-synuclein gene in patients with Parkinson's disease. Mov Disord 16:1115–1119

Payami H, Larsen K, Bernard S, Nutt J (1994) Increased risk of Parkinson's disease in parents and siblings of patients. Ann Neurol 36:659–661

Piccini P, Morrish PK, Turjanski N, Sawle GV, Burn DJ, Weeks RA, Mark MH, Maraganore DM, Lees AJ, Brooks DJ (1997) Dopaminergic function in familial Parkinson's disease: a clinical and 18F-dopa positron emission tomography study. Ann Neurol 41:222–229

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047

Preux PM, Condet A, Anglade C, Druet-Cabanac M, Debrock C, Macharia W, Couratier P, Boutros-Toni F, Dumas M (2000) Parkinson's disease and environmental factors: matched case-control study in the Limousin region, France. Neuroepidemiology 19:333–337

Riordan SM, Williams R (2001) The Wilson's disease gene and phenotypic diversity. J Hepatol 34:165–171

Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, Lyons K, Pahwa R, et al (2001) Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 286: 2239–2244

Siemers ER, Hubble J, Tuite P, Comella C, Kompoliti K, Oakes D, Wojcieszek J, Foroud T, Conneally PM (1998) Clinical diagnostic criteria for idiopathic Parkinson's disease. Mov Disord 13:862

Sveinbjornsdottir S, Hicks AA, Jonsson T, Petursson H, Gugmundsson G, Frigge ML, Kong A, Gulcher JR, Stefansson K (2000) Familial aggregation of Parkinson's disease in Iceland. N Engl J Med 343:1765–1770

Tanner CM, Ottmann R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW (1999) Parkinson's disease in twins: an etiologic study. JAMA 281:341–346

Tanner CM, Thelen JA, Oxford KP, Rademacher D, Goetz CG, Kurland LT (1992) Parkinson's disease incidence in Olmstead County, MN: 1935–1988. Neurology 42 Suppl 3: 194

Uitti RJ, Shinotoh H, Hayward M, Schulzer M, Mak E, Calne DB (1997) Familial Parkinson's disease: a case-control study of families. Can J Neurol Sci 24:127–132

Uversky VN, Li J, Fink AL (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein: a possible molecular NK between Parkinson's disease and heavy metal exposure. J Biol Chem 276: 44284–44296

Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, Albanese A, Wood NW (2001) Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on chromosome 1p35-p36. Am J Hum Genet 68:895–900

van Duijn CM, Dekker MCJ, Bonifati V, Galjaard RJ, Houwing-Duistermaat JJ, Snijders PJLM, Testers L, Breedveld GJ, Horstink M, Sandkuijl LA, van Swieten JC, Oostra BA, Heutink P (2001) PARK7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am J Hum Genet 69:629–634

Vaughan JR, Farrer MJ, Wszolek ZK, Gasser T, Durr A, Agid Y, Bonifati V, DeMichele G, Volpe G, Lincoln S, Breteler M, Meco G, Brice A, Marsden CD, Hardy J, Wood NW (1998) Sequencing of the alpha-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations: The European Consortium on Genetic Susceptibility in Parkinson's Disease (GSPD). Hum Mol Genet 7:751–753

Vieregge P, Friedrich HJ, Rohl A, Ulm G, Heberlein I (1994) [Multifactorial etiology of idiopathic Parkinson disease: a case-control study]. Nervenarzt 65:390–395 (in German)

Yamamura Y, Sobue I, Ando K, Iida M, Yanagi T, Kondo C (1973) Paralysis agitans of early-onset with marked diurnal fluctuation of symptoms. Neurology 23:239–244