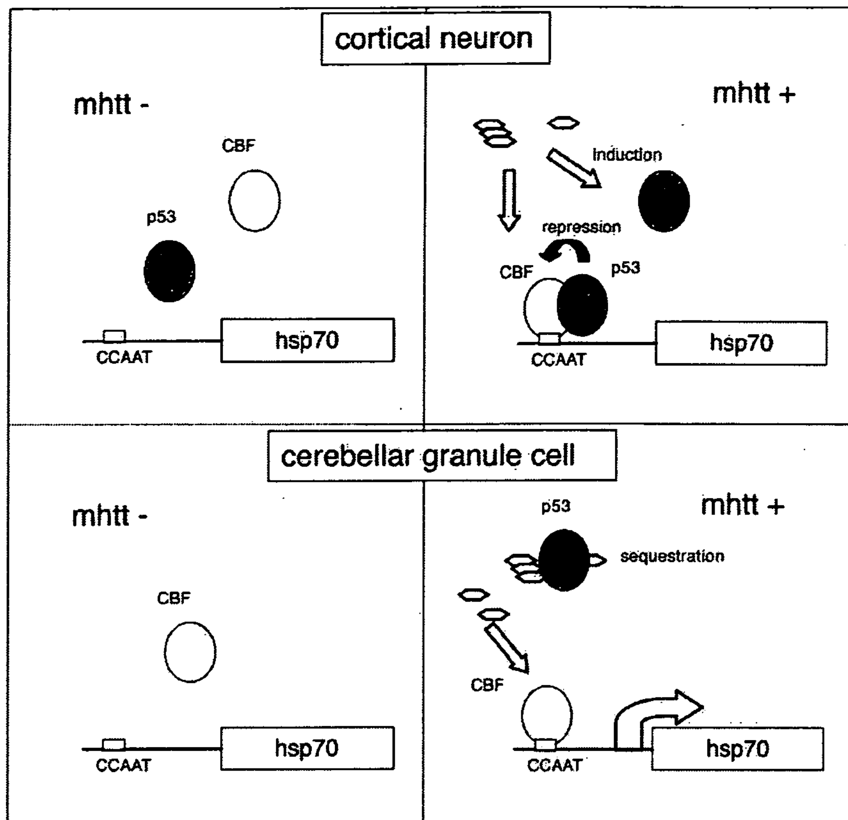

Figure 10. A hypothetical scheme for transcriptional regulation of *hsp70* in cortical neurons and cerebellar neurons. The basal expression level of p53 is higher in cortical neurons than in cerebellar neurons (top and bottom left). In the presence of mutant htt (mhtt), CBF binds to the *hsp70* gene promoter in cortical and cerebellar neurons (top and bottom right), whereas p53 induced by mutant htt represses CBF in cortical neurons (top right). In contrast, p53 is not induced in most of the cerebellar neurons in which suppression by p53 does not work (bottom right). Although p53 is induced in a small part of cerebellar neurons, it is sequestered into inclusion bodies.

subtype-specific response to p53. The mechanistic knowledge could be useful for developing a novel therapeutic approach where vulnerable neurons are changed to resistant neurons in the HD pathology.

References

Adachi H, Katsuno M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, Kusakabe M, Yoshiki A, Kobayashi Y, Doyu M, Sobue G (2003) Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci 23:2203–2211.

Agoff SN, Hou J, Linzer DI, Wu B (1993) Regulation of the human hsp70 promoter by p53. Science 259:84–87.

Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810.

Bae BI, Xu H, Igarashi S, Fujimuro M, Agrawal N, Taya Y, Hayward SD, Moran TH, Montell C, Ross CA, Snyder SH, Sawa A (2005) p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. Neuron 47:29–41.

Chai Y, Koppenhafer SL, Bonini NM, Paulson HL (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19:10338–10347.

Chan EY, Luthi-Carter R, Strand A, Solano SM, Hanson SA, DeJohn MM, Kooperberg C, Chase KO, DiFiglia M, Young AB, Leavitt BR, Cha JH, Aronin N, Hayden MR, Olson JM (2002) Increased huntingtin protein length reduces the number of polyglutamine-induced gene expression changes in mouse models of Huntington's disease. Hum Mol Genet 11:1939–1951.

Colin E, Regulier E, Perrin V, Durr A, Brice A, Aebischer P, Deglon N, Humbert S, Saudou F (2005) Akt is altered in an animal model of Huntington's disease and in patients. Eur J Neurosci 21:1478–1488.

Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19:148–154.

Cummings CJ, Sun Y, Opal P, Antalffy B, Mestril R, Orr HT, Dilmann WH, Zoghbi HY (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum Mol Genet 10:1511–1518.

Engqvist-Goldstein AE, Kessels MM, Chopra VS, Hayden MR, Drubin DG (1999) An actin-binding protein of the Sla2/Huntingtin interacting protein 1 family is a novel component of clathrin-coated pits and vesicles. J Cell Biol 147:1503–1518.

Feng Z, Jin S, Zupnick A, Hoh J, de Stanchina E, Lowe S, Prives C, Levine AJ (2006) p53 tumor suppressor protein regulates the level of huntingtin gene expression. Oncogene 25:1–7.

Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. Science 311:1471–1474.

Hazeki N, Tsukamoto T, Yazawa I, Koyama M, Hattori S, Someki I, Iwatsubo T, Nakamura K, Goto J, Kanazawa I (2002) Ultrastructure of nuclear aggregates formed by expressing an expanded polyglutamine. Biochem Biophys Res Commun 294:429–440.

Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, et al. (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15:965–977.

Hoshino M, Tagawa K, Okuda T, Murata M, Oyanagi K, Arai N, Mizutani T, Kanazawa I, Wanker EE, Okazawa H (2003) Histone deacetylase activity is retained in primary neurons expressing mutant huntingtin protein. J Neurochem 87:257–267.

Hoshino M, Tagawa K, Okuda T, Okazawa H (2004) General transcriptional repression by polyglutamine disease proteins is not directly linked to the presence of inclusion bodies. Biochem Biophys Res Commun 313:110–116.

Hoshino M, Qi ML, Yoshimura N, Miyashita T, Tagawa K, Wada Y, Enokido Y, Marubuchi S, Harjes P, Arai N, Oyanagi K, Blandino G, Sudol M, Rich T, Kanazawa I, Wanker EE, Saitoe M, Okazawa H (2006) Transcriptional repression induces a slowly progressive atypical neuronal death associated with changes of YAP isoforms and p73. J Cell Biol 172:589–604.

Humbert S, Saudou F (2002) Toward cell specificity in SCA1. Neuron 30:669–670.

Humbert S, Bryson EA, Cordelieres FP, Connors NC, Datta SR, Finkbeiner S, Greenberg ME, Saudou F (2002) The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. Dev Cell 2:831–837.

Jana NR, Tanaka M, Wang G, Nukina N (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum Mol Genet 9:2009–2018.

Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z, Davies E, Hajnoczky G, Saunders TL, Van Keuren ML, Fernandes-Alnemri T, Meisler MH, Alnemri ES (2003) Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. Nature 425:721–727.

Kao HT, Capasso O, Heintz N, Nevins JR (1985) Cell cycle control of the human HSP70 gene: implications for the role of a cellular E1A-like function. Mol Cell Biol 5:628–633.

Kim M, Velier J, Chase K, Laforet G, Kalchman MA, Hayden MR, Won L, Heller A, Aronin N, Difiglia M (1999) Forskolin and dopamine D1 receptor activation increases huntingtin's association with endosomes in immortalized neuronal cells of striatal origin. Neuroscience 89:1159–1167.

Krobitsch S, Lindquist S (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. Proc Natl Acad Sci USA 97:1589–1594.

Legendre-Guillemin V, Metzler M, Lemaire JF, Philie J, Gan L, Hayden MR, McPherson PS (2005) Huntingtin interacting protein 1 (HIP1) regulates clathrin assembly through direct binding to the regulatory region of the clathrin light chain. J Biol Chem 280:6101–6108.

Li H, Li SH, Johnston H, Shelbourne PF, Li XJ (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. Nat Genet 25:385–389.

Luthi-Carter R, Hanson SA, Strand AD, Bergstrom DA, Chun W, Peters NL, Woods AM, Chan EY, Kooperberg C, Krainc D, Young AB, Tapscott SJ, Olson JM (2002a) Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum Mol Genet 11:1911–1926.

Luthi-Carter R, Strand AD, Hanson SA, Kooperberg C, Schilling G, La Spada AR, Merry DE, Young AB, Ross CA, Borchelt DR, Olson JM (2002b) Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington's disease mouse models reveal context-independent effects. Hum Mol Genet 11:1927–1937.

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506.

Matilla A, Koshy BT, Cummings CJ, Isobe T, Orr HT, Zoghbi HY (1997) The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. Nature 389:974–978.

Metzler M, Legendre-Guillemin V, Gan L, Chopra V, Kwok A, McPherson PS, Hayden MR (2001) HIP1 functions in clathrin-mediated endocytosis through binding to clathrin and adaptor protein 2. J Biol Chem 276:39271–39276.

Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, Hartl FU (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. Proc Natl Acad Sci USA 97:7841–7846.

Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H (1990) A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell 60:461–472.

Okazawa H (2003) Polyglutamine diseases: a transcription disorder? Cell Mol Life Sci 60:1427–1439.

Okazawa H, Okamoto K, Ishino F, Ishino-Kaneko T, Takeda S, Toyoda Y, Muramatsu M, Hamada H (1991) The oct3 gene, a gene for an embryonic transcription factor, is controlled by a retinoic acid repressible enhancer. EMBO J 10:2997–3005.

Okazawa H, Rich T, Chang A, Lin X, Waragai M, Kajikawa M, Enokido Y, Komuo A, Kato S, Shibata M, Hatanaka H, Mouradian MM, Sudol M, Kanazawa I (2002) Interaction between mutant ataxin-1 and PQBP-1 affects transcription and cell death. Neuron 34:701–713.

Pardo P, Colin E, Regulier E, Aebischer P, Deglon N, Humbert S, Saudou F (2006) Inhibition of calcineurin by FK506 protects against polyglutamine-huntingtin toxicity through an increase of huntingtin phosphorylation at S421. J Neurosci 26:1635–1645.

Peschard P, Park M (2003) Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. Cancer Cell 3:519–523.

Pirkkala L, Nykanen P, Sistonen L (2001) Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J 15:1118–1131.

Rangone H, Poizat G, Troncoso J, Ross CA, MacDonald ME, Saudou F, Humbert S (2004) The serum- and glucocorticoid-induced kinase SGK inhibits mutant huntingtin-induced toxicity by phosphorylating serine 421 of huntingtin. Eur J Neurosci 19:273–279.

Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, Parlow AF, Ross TS (2001) Huntingtin interacting protein 1 is a clathrin coat binding protein required for differentiation of late spermatogenic progenitors. Mol Cell Biol 21:7796–7806.

Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*. Cell 90:549–558.

Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852.

Sipione S, Rigamonti D, Valenza M, Zuccato C, Conti L, Pritchard J, Kooperberg C, Olson JM, Cattaneo E (2002) Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Hum Mol Genet 11:1953–1965.

Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci USA 97:6763–6768.

Strand AD, Aragaki AK, Shaw D, Bird T, Holton J, Turner C, Tapscott SJ, Tabrizi SJ, Schapira AH, Kooperberg C, Olson JM (2005) Gene expression in Huntington's disease skeletal muscle: a potential biomarker. Hum Mol Genet 14:1863–1876.

Strauss KM, Martins LM, Plun-Favreau H, Marx FP, Kautzmann S, Berg D, Gasser T, Wszolek Z, Muller T, Bornemann A, Wolburg H, Downward J, Riess O, Schulz JB, Kruger R (2005) Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum Mol Genet 14:2099–2111.

Tagawa K, Hoshino M, Okuda T, Ueda H, Hayashi H, Engemann S, Okado H, Ichikawa M, Wanker EE, Okazawa H (2004) Distinct aggregation and cell death patterns among different types of primary neurons induced by mutant huntingtin protein. J Neurochem 89:974–987.

Vitour D, Lindenbaum P, Vende P, Becker MM, Poncet D (2004) RoXaN, a novel cellular protein containing TPR, LD, and zinc finger motifs, forms a ternary complex with eukaryotic initiation factor 4G and rotavirus NSP3. J Virol 78:3851–3862.

Wacker JL, Zareie MH, Fong H, Sarikaya M, Muchowski PJ (2004) Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. Nat Struct Mol Biol 11:1215–1222.

Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, Wanker EE (2001a) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol Biol Cell 12:1393–1407.

Waelter S, Scherzinger E, Hasenbank R, Nordhoff E, Lurz R, Goehler H, Gauss C, Sathasivam K, Bates GP, Lehrach H, Wanker EE (2001b) The huntingtin interacting protein HIP1 is a clathrin and alpha-adaptin-binding protein involved in receptor-mediated endocytosis. Hum Mol Genet 10:1807–1817.

Waragai M, Claas-Hinrich L, Takeuchi S, Imafuku I, Udagawa Y, Kanazawa I, Kawabata M, Mouradian MM, Okazawa H (1999) PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. Hum Mol Genet 8:977–987.

Warby SC, Chan EY, Metzler M, Gan L, Singaraja RR, Crocker SF, Robertson HA, Hayden MR (2005) Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. Hum Mol Genet 14:1569–1577.

Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. Nat Genet 23:425–428.

Williams GT, McClanahan TK, Morimoto RI (1989) E1a transactivation of the human HSP70 promoter is mediated through the basal transcriptional complex. Mol Cell Biol 9:2574–2587.

Wu BJ, Hurst HC, Jones NC, Morimoto RI (1986) The E1A 13S product of adenovirus 5 activates transcription of the cellular human HSP70 gene. Mol Cell Biol 6:2994–2999.

Wyttenbach A, Carmichael J, Swartz J, Furlong RA, Narain Y, Rankin J, Rubinsztein DC (2000) Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci USA 97:2898–2903.

Wyttenbach A, Swartz J, Kita H, Thykjaer T, Carmichael J, Bradley J, Brown R, Maxwell M, Schapira A, Orntoft TF, Kato K, Rubinsztein DC (2001) Polyglutamine expansions cause decreased CRE-mediated transcription and early gene expression changes prior to cell death in an inducible cell model of Huntington's disease. Hum Mol Genet 10:1829–1845.

Zhou H, Li SH, Li XJ (2001) Chaperone suppression of cellular toxicity of huntingtin is independent of polyglutamine aggregation. J Biol Chem 276:48417–48424.

Zylicz M, King FW, Wawrzynow A (2001) Hsp70 interactions with the p53 tumor suppressor protein. EMBO J 20:4634–4638.

Fibroblast growth factor 20 gene and Parkinson's disease in the Japanese population

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Received 3 January 2007; accepted 20 February 2007

A genetic association between the fibroblast growth factor 20 (*FGF20*) gene and Parkinson's disease has been found by the pedigree disequilibrium test. This association, however, was not replicated by a case–control association study. In order to clarify the association between the *FGF20* gene and Parkinson's disease, we attempted to replicate this association by a case–control association study using a large number of Japanese samples (1388 patients and 1891 controls). rs1721100 exhibited a significant difference in allele C versus G (P=0.0089), and in genotype CC+CG versus GG (P=0.0053). Haplotype association analysis showed that haplotype 2 was the protective haplotype for Parkinson's disease (permutation-P=0.0075). These results suggest that the *FGF20* gene is a susceptibility gene for Parkinson's disease in the Japanese population. *NeuroReport* 18:937–940 © 2007 Lippincott Williams & Wilkins.

Keywords: association, case–control study, fibroblast growth factor 20 (*FGF20*), Parkinson's disease, single nucleotide polymorphism, susceptibility gene

Introduction

Parkinson's disease (OMIM #168600) is one of the most common neurodegenerative diseases, characterized by resting tremor, cogwheel rigidity, bradykinesia, and impaired postural reflexes. These clinical features result primarily from the loss of dopaminergic neurons in the substantia nigra. Various medical treatments improve Parkinson's disease symptoms, but do little to deter disease progression [1]. In Mendelian-inherited Parkinson's disease, eight causal genes have been identified (*SNCA*, *parkin*, *UCHL1*, *PINK1*, *DJ1*, *LRRK2/dardarin*, *ATP13A2*, and *NR4A2/Nurr1*). Sporadic Parkinson's disease is a complex disorder, with multiple genetic and environmental factors influencing disease risk [2]. Identifying genetic risk factors for Parkinson's disease will be helpful in elucidating the pathogenesis of Parkinson's disease.

Genome-wide, non-parametric linkage analyses of Parkinson's disease families have revealed significant linkage in multiple chromosomal regions [3–6]. One of these prominent regions of linkage was found on chromosome 8p (LOD score 2.2 at D8S520) [6]. Subsequently, van der Walt *et al.* chose to examine the *FGF20* gene in their investigation of biological candidate genes for Parkinson's disease susceptibility in this region. The *FGF20* gene is approximately 9.3 kb (*http://genome.ucsc.edu/*), and is located approximately 6.2 Mb from a peak marker D8S520 on chromosome 8p22–p21.3 [7]. FGF20 is a neurotrophic factor that exerts strong neurotrophic properties within brain tissue, and regulates central nervous development and function [8]. FGF20 is preferentially expressed in the substantia nigra [9], and it has been reported to be involved in dopaminergic neurons survival [10]. In order to assess the genetic association of the *FGF20* gene with Parkinson's disease, they genotyped five single nucleotide polymorphisms (SNPs) [ss20399076 (rs12718379), rs1989756, rs1989754, rs1721100, and ss20399075 (rs12720208)] lying within the *FGF20* gene in 644 families from the United States, performed the pedigree disequilibrium test (PDT), the genotype PDT, the multilocus-genotype PDT, and the family-based association test, and discovered a highly significant association of Parkinson's disease with one intronic SNP, rs1989754 (P=0.0006), and two SNPs, rs1721100 (P=0.02) and rs12720208 (P=0.0008), located in the 3′ regulatory region. Furthermore, they detected a haplotype that is positively associated with risk of Parkinson's disease (P=0.0003), whereas a second haplotype was

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found to be negatively associated with risk of Parkinson's disease (P=0.0009). Consequently, they concluded that the *FGF20* gene was a susceptibility gene for Parkinson's disease [11].

Subsequently, Clarimon *et al.* sought to replicate the association of the *FGF20* gene with Parkinson's disease by performing a case–control association study with four SNPs [rs1989756, rs1989754, rs1721100, and ss20399075 (rs12720208)] using Finnish and Greek samples. They found a difference in allele frequency in only rs1989754, but the difference was not significant after the Bonferroni correction. They also found no significant difference in the distribution of haplotypes between patients and controls. They hence failed to replicate the association of the *FGF20* gene with Parkinson's disease [12]. Thus, it is still controversial as to whether the *FGF20* gene is a susceptibility gene for Parkinson's disease or not. We here conducted a case–control association study using a large number of Japanese samples in order to evaluate the association of the *FGF20* gene with risk of Parkinson's disease.

Materials and methods

We recruited 1388 unrelated Parkinson's disease patients (age, 65.7±9.8; male/female ratio, 0.84) and 1891 unrelated controls (age, 48.5±17.6; male/female ratio, 1.08). The diagnosis of Parkinson's disease was based on the presence of two or more of the cardinal features of Parkinson's disease (tremor, rigidity, bradykinesia, and postural instability), according to the criteria for Parkinson's disease [13]. Patients were evaluated by certified neurologists specializing in Parkinson's disease. The average age of onset was 57.7±11.1 years. All patients and controls were of Japanese ancestry. Informed consent was obtained from each individual, and approval for the study was obtained from the University Ethical Committees. Genomic DNA was extracted from venous blood using standard procedures.

The TaqMan SNP Genotyping Assay (Applied Biosystems, Foster, California, USA) was employed for five SNPs (rs12718379, rs1989756, rs1989754, rs1721100, and rs12720208). SNP information was obtained from the dbSNP database (*http://www.ncbi.nlm.nih.gov/SNP/*) and the International HapMap Project database (*http://hapmap.org*) [14].

All statistical analyses were performed by using the software SNPAlyze (Dynacom, Japan). Genotype deviation from Hardy–Weinberg equilibrium was assessed by the χ^2 test. The statistical significance of a case–control association was evaluated by the χ^2 test, and odds ratio and its 95% confidence intervals (CIs) were calculated by the Bootstrap method. Haplotype frequencies were estimated using an expectation-maximization algorithm [15]. We evaluated pair-wise linkage disequilibrium (LD) among SNPs by D' value, and r^2 as standards for LD. Case–control haplotype analyses were carried out by calculating the permutation P-value on the basis of 10 000 replications [16].

Results

Two SNPs (rs1989756 and rs12720208) of the five SNPs, examined by van der Walt *et al.*, showed a monomorphism in 95 individuals drawn from the Japanese population, and therefore these two SNPs were excluded from further analysis. Three SNPs (rs1989754, rs1721100, and

Table 1 Summary of the association of three SNPs between Parkinson's disease patients and controls

SNP ID	Alleles		Genotype										Allele 1 versus Allele 2		Genotype 11 versus 12+22		Genotype 11+12 versus 22	
			Patient					Control										
	1–2	Strand[a]	1/1	1/2	2/2	Total	Frequency of allele 1	1/1	1/2	2/2	Total	Frequency of allele 1	Odds ratio (95% CI[b])	P-value	Odds ratio (95% CI[b])	P-value	Odds ratio (95% CI[b])	P-value
rs12718379	A-G	Reverse	249	641	481	1371	0.42	375	902	597	1874	0.44	1.11 (1.01–1.23)	0.041	1.13 (0.95–1.34)	0.19	1.16 (1.00–1.34)	0.054
rs1989754	G-C	Forward	261	628	477	1366	0.42	381	895	586	1862	0.44	1.10 (1.00–1.22)	0.055	1.09 (0.92–1.28)	0.34	1.17 (1.01–1.35)	0.040
rs1721100	C-G	Forward	270	639	458	1367	0.43	407	925	542	1874	0.46	1.14 (1.03–1.26)	0.0089	1.13 (0.94–1.36)	0.17	1.24 (1.06–1.43)	0.0053

[a]Relative to the transcriptional direction.
[b]CI, confidence intervals; SNP, single nucleotide polymorphism.

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rs12720208) are included in HapMap. rs12720208 also shows no polymorphism in JPT (Japanese in Tokyo) HapMap, consistent with the genotyping results of our samples. In the *FGF20* gene region, 171 SNPs were observed in dbSNP. According to JPT HapMap, the Tagger method showed that two SNPs (rs1989754 and rs1721100) can represent the remainder of the HapMap SNPs of the *FGF20* gene region, as tag SNPs with a criteria of $r^2>0.8$ and a minor allele frequency >0.1, although the number of tag SNPs differed between JPT and CEU [CEPH (Utah residents with ancestry from northern and western Europe)] [17]. Thus, we considered that a case–control association study using three SNPs [two tag SNPs (rs1989754 and rs1721100) plus rs12718379] was appropriate for assessing the association of the *FGF20* gene with Parkinson's disease.

Table 1 shows the results of the SNP genotyping in the Parkinson's disease patients and controls. The association of rs1721100 was significant in allele 1 versus allele 2 [frequency of allele 1; 43% in patients and 46% in controls, $P=0.0089$, odds ratio 1.14 (95% CI, 1.03–1.26)] and in genotype 11+12 versus 22 [$P=0.0053$, odds ratio 1.24 (95% CI, 1.06–1.43)]. The association with rs1721100 was significant even after the Bonferroni correction (tests for three SNPs). As for rs12718379, a decrease in frequency of allele 1 was found in patients compared with controls [frequency of allele 1; 42% in patients and 44% in controls, $P=0.041$, odds ratio 1.11 (95% CI, 1.01–1.23)]. As for rs1989754, there was a difference in frequency of genotype 11+12 versus 22 between patients and controls [$P=0.040$, odds ratio 1.17 (95% CI, 1.01–1.35)]. Neither rs12718379 nor rs1989754, however, showed a significant association with Parkinson's disease after the Bonferroni correction. The genotype frequencies of all three SNPs were not significantly different from the values expected from the Hardy–Weinberg equilibrium.

We calculated the LD among the three SNPs in patients and controls. D' values (absolute value) and r^2 for pair-wise LD of controls are shown in Table 2. A high LD was detected between each pair of SNPs, and the same trend was observed in patients and in the JPT samples of the HapMap database (data not shown). These findings suggested that the three SNPs were in single LD, and we therefore performed haplotype association analysis. Haplotype frequencies of the three SNPs were estimated in patients and controls (Table 3). Two common haplotypes (haplotypes 1 and 2) covered >90% of the population haplotypes in both patients and controls. The frequency of haplotype 2 (A-G-C) was significantly less in patients than controls (38% in patients and 41% in controls, permutation-$P=0.0075$). This indicates that haplotype 2 is a protective haplotype for Parkinson's disease in the Japanese population. Taken together, our genetic analyses support the *FGF20* gene being a susceptibility gene for Parkinson's disease in the Japanese population.

Table 2 Linkage disequilibrium between SNPs in the *FGF20* gene

SNP ID	rs12718379	rs1989754	rs1721100
rs12718379	—		
rs1989754	0.94 (0.98)	—	
rs1721100	0.68 (0.86)	0.72 (0.88)	—

r^2 (D') values of controls are shown for each pair of single nucleotide polymorphisms (SNPs).

Discussion

Our results are consistent with the report by van der Walt *et al.* [11], which showed an association of the *FGF20* gene with risk of Parkinson's disease. The significance of the *FGF20* gene for Parkinson's disease susceptibility in our study, however, was not so strong as that shown by van der Walt *et al.* This discrepancy may result from: (i) the ethnic differences between the Japanese samples and samples from the United States; the association in the Japanese population might be smaller than in the United States, or (ii) the difference in epidemiological approaches; we performed a case–control association study by the χ^2 test in unrelated samples, while they analysed family-based samples by the PDT. rs12720208, the strongly associated SNP in the report by van der Walt *et al.*, was excluded from our study because we were not able to find polymorphism of this SNP in the Japanese samples. It is interesting that rs1721100, the most strongly associated SNP in our study, and rs12720208, however, are both located in the 3′ UTR region of the *FGF20* gene. LD indices between rs12720208 and rs1721100 showed that these two SNPs are in a single LD block ($D'=1$) and that the correlation was not strong ($r^2=0.28$) (on the basis of CEU HapMap).

On the other hand, the case–control association study by Clarimon *et al.* [12] failed to replicate the association of the *FGF20* gene with risk of Parkinson's disease, although the rs1989754 G allele frequency was higher in patients than controls in the Finnish samples (52% in patients and 42% in controls, $P=0.03$ before Bonferroni correction). However, as their sample size was not large enough, their study does not disprove the association of the *FGF20* gene with Parkinson's disease convincingly if the influence for Parkinson's disease in the Greek and Finnish population is to the same extent as in our Japanese sample. The sample size of their study was considerably smaller than ours (Finnish series, 144 patients and 135 controls; Greek series, 151 patients and 186 controls in their study, compared with 1388 patients and 1891 controls in our study). As mentioned in their report, their experiment had 80% power to detect

Table 3 Haplotype association analysis using three SNPs in the *FGF20* gene

	Base at SNP			Haplotype frequency		
Haplotype ID	rs12718379	rs1989754	rs1721100	Patient	Control	*P*-value
Haplotype 1	G	C	G	0.53	0.50	0.054
Haplotype 2	A	G	C	0.38	0.41	0.0075
Haplotype 3	G	C	C	0.045	0.047	0.75
Haplotype 4	A	G	G	0.035	0.028	0.11

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risks from 1.7 to 3.6 in the Finnish samples and from 1.6 to 2.1 in the Greek samples, whereas the odds ratio of the *FGF20* gene in our data was 1.14. The possibility of type 2 errors in their study could not be excluded as an explanation for this negative finding. Another explanation for lack of replication could be genetic heterogeneity; there might not be an association between the *FGF20* gene and Parkinson's disease in the Greek and Finnish populations, whereas there might be in the Japanese and the United States-based population.

In this study, the three SNPs (rs12718379, rs1989754, and rs1721100) showed a difference between patients and controls to some degree. After Bonferroni correction, however, a significant association was detected only in rs1721100. The correlations between rs1721100 and the other two SNPs were not strong (r^2=0.68 with rs12718379 and 0.72 with rs1989754), which might explain the different extents of significance among the three SNPs.

The *EFHA2* gene is located 25 kb upstream of the 5′ UTR, and the *MSR1* gene is located 800 kb downstream of the *FGF20* gene. The entire region of the *FGF20* gene is within a single LD block of 20.8 kb (on the basis of JPT HapMap). Moreover, no other known genes reside within this LD block. Therefore, we concluded that our positive finding results from the association between the *FGF20* gene and Parkinson's disease.

Conclusion

We performed a case–control association study using a large number of samples (1388 Parkinson's disease patients and 1891 controls) in the Japanese population, and found a significant association of Parkinson's disease with rs1721100 and haplotype 2 (A-G-C) in the *FGF20* gene. Our results, together with those of van der Walt *et al.*, demonstrate an association of the *FGF20* gene with Parkinson's disease in two different ethnic groups. This evidence suggests the involvement of the *FGF20* gene in the pathogenesis of Parkinson's disease.

Acknowledgements

The authors are grateful to the Parkinson's disease patients who participated in this study. The authors thank Drs Akira Oka, Hidetoshi Inoko, and Katsushi Tokunaga for control samples. They also thank Dr Helena A. Popiel for editing the manuscript. This work was supported by the 21st century COE program and Research Grants (17019044 and 17590874), both from the Ministry of Education, Culture, Sports, Science and Technology of Japan; by the Grant for Research on Measures for Intractable Diseases (H17-Q-15-1) from the Ministry of Health, Labor and Welfare of Japan; and by a grant from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency (JST).

References

1. Rascol O, Payoux P, Ory F, Ferreira JJ, Brefel-Courbon C, Montastruc JL. Limitations of current Parkinson's disease therapy. *Ann Neurol* 2003; 53:S3–S12.
2. Warner TT, Schapira AH. Genetic and environmental factors in the cause of Parkinson's disease. *Ann Neurol* 2003; 53:S16–S23.
3. DeStefano AL, Golbe LI, Mark MH, Lazzarini AM, Maher NE, Saint-Hilaire M, *et al.* Genome-wide scan for Parkinson's disease: the GenePD study. *Neurology* 2001; 57:1124–1126.
4. Hicks AA, Petursson H, Jonsson T, Stefansson H, Johannsdottir HS, Sainz J, *et al.* A susceptibility gene for late-onset idiopathic Parkinson's disease. *Ann Neurol* 2002; 52:549–555.
5. Pankratz N, Nichols WC, Uniacke SK, Halter C, Rudolph A, Shults C, *et al.* Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. *Am J Hum Genet* 2002; 71:124–135.
6. Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, Lyons K, *et al.* Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA* 2001; 286:2239–2244.
7. Kirikoshi H, Sagara N, Saitoh T, Tanaka K, Sekihara H, Shiokawa K, *et al.* Molecular cloning and characterization of human FGF-20 on chromosome 8 p21.3-p22. *Biochem Biophys Res Commun* 2000; 274:337–343.
8. Jeffers M, Shimkets R, Prayaga S, Boldog F, Yang M, Burgess C, *et al.* Identification of a novel human fibroblast growth factor and characterization of its role in oncogenesis. *Cancer Res* 2001; 61:3131–3138.
9. Ohmachi S, Watanabe Y, Mikami T, Kusu N, Ibi T, Akaike A, *et al.* FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain. *Biochem Biophys Res Commun* 2000; 277:355–360.
10. Ohmachi S, Mikami T, Konishi M, Miyake A, Itoh N. Preferential neurotrophic activity of fibroblast growth factor-20 for dopaminergic neurons through fibroblast growth factor receptor-1c. *J Neurosci Res* 2003; 72:436–443.
11. Van der Walt JM, Noureddine MA, Kittappa R, Hauser MA, Scott WK, McKay R, *et al.* Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. *Am J Hum Genet* 2004; 74:1121–1127.
12. Clarimon J, Xiromerisiou G, Eerola J, Gourbali V, Hellstrom O, Dardiotis E, *et al.* Lack of evidence for a genetic association between FGF20 and Parkinson's disease in Finnish and Greek patients. *BMC Neurol* 2005; 5:11.
13. Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976–1990. *Neurology* 1999; 52:1214–1220.
14. The International HapMap Consortium. The International HapMap Project. *Nature* 2003; 426:789–796.
15. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 1995; 12: 921–927.
16. Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, *et al.* Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 2001; 11:143–151.
17. De-Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005; 37:1217–1223.

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J Hum Genet (2007) 52:643–649
DOI 10.1007/s10038-007-0154-1

ORIGINAL ARTICLE

Redefining the disease locus of 16q22.1-linked autosomal dominant cerebellar ataxia

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Received: 31 January 2007 / Accepted: 2 May 2007 / Published online: 5 July 2007
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Abstract The 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA; Online Mendelian Inheritance in Man [OMIN] #117210) is one of the most common ADCAs in Japan. Previously, we had reported that the patients share a common haplotype by founder effect and that a C-to-T substitution (–16C>T) in the *puratrophin*-1 gene was strongly associated with the disease. However, recently, an exceptional patient without the substitution was reported, indicating that a true pathogenic mutation might be present elsewhere. In this study, we clarified the disease locus more definitely by the haplotype analysis of families showing pure cerebellar ataxia. In addition to microsatellite markers, the single nucleotide polymorphisms (SNPs) that we identified on the disease chromosome were examined to confirm the borders of the disease locus. The analysis of 64 families with the –16C>T substitution in the *puratrophin*-1 gene revealed one family showing an ancestral recombination event between SNP04 and SNP05 on the disease chromosome. The analysis of 22 families without identifiable genetic mutations revealed another family carrying the common haplotype centromeric to the *puratrophin*-1 gene, but lacking the –16C>T substitution in this gene. We concluded that the disease locus of 16q-ADCA was definitely confined to a 900-kb genomic region between the SNP04 and the –16C>T substitution in the *puratrophin*-1 gene in 16q22.1.

Keywords 16q-ADCA · Pure cerebellar ataxia · Haplotype · SNP · Founder effect · SCA4

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Introduction

Autosomal dominant cerebellar ataxia (ADCA) is a clinical entity of heterogeneous neurodegenerative diseases that show dominantly inherited, progressive cerebellar ataxia that can be variably associated with other neurological and systemic features (Harding 1982). ADCA is now classified by the responsible mutations or gene loci. Subtypes of ADCA of which causative genes or gene loci have been identified are known as spinocerebellar ataxia type (SCA) 1, 2, 3 (or Machado-Joseph disease), 4–8, 10–19, 21–23, 25, 26, 28, dentatorubral and pallidoluysian atrophy (DRPLA), and ADCA with mutation in the fibroblast growth factor (FGF) 14 gene (Schöls et al. 2004; Yu et al. 2005; Cagnoli et al. 2006).

Among these, mutations in SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and DRPLA have been identified as

the expansions of a trinucleotide (CAG) repeat that encodes the polyglutamine tract, uniformly causing the aggregation of polyglutamine-containing causative protein (Ross and Poirier 2004). The expansion of noncoding trinucleotide (CAG or CTG) or pentanucleotide (ATTCT) repeats are involved in SCA8, SCA10, and SCA12 (Holmes et al. 1999; Koob et al. 1999; Matsuura et al. 2000). Very few families are affected by missense mutations in beta-III spectrin (*SPTBN2*) (SCA5 (see Ikeda et al. 2006)), voltage-gated potassium channel KCNC3 (SCA13 (see Waters et al. 2006)), protein kinase C gamma (PKC gamma) (SCA14 (see Chen et al. 2003)), and *FGF14* genes (ADCA with FGF14 mutation (see van Swieten et al. 2003). However, genes or even loci remain unidentified for 20–40% of families with ADCA (Sasaki et al. 2003).

We had previously found that Japanese families with ADCA map to the human chromosome 16q22.1 (16q-ADCA), the gene locus of SCA4 (Flanigan et al. 1996; Hellenbroich et al. 2005; Nagaoka et al. 2000). However, our families show clinically pure cerebellar ataxia without other neurological signs, such as sensory neuropathy or pyramidal tract signs seen in SCA4. All 16q-ADCA patients shared a common haplotype, presumably due to inheritance from a disease chromosome of a founder (Takashima et al. 2001). Our haplotype analysis of 52 families with DNA polymorphic microsatellite markers revealed that they all share a common haplotype for the 400-kb genomic region in 16q22.1 (Ishikawa et al. 2005). Within this region, we found that a heterozygous single nucleotide C-to-T substitution (–16C>T) in the untranslated region of the *puratrophin*-1 gene was entirely segregated with all patients, suggesting a strong association with the disease. This substitution was also found in other cohorts of Japanese families with ataxia (Ouyang et al. 2006; Onodera et al. 2006), while it was not found in Caucasian patients in Europe (Wieczorek et al. 2006). The frequency of 16q-ADCA is considered to be relatively high in Japan, counted as the third or fourth major subtype of ADCA after MJD, SCA6, and DRPLA (Takano et al. 1998; Sasaki et al. 2003; Ohata et al. 2006).

However, one group recently reported an exceptional patient without the –16C>T substitution in the *puratrophin*-1 gene, in a family in which all of the other affected subjects carried the substitution (Ohata et al. 2006). This patient shared the common haplotype in a region centromeric to the substitution in the *puratrophin*-1 gene, suggesting that a true pathogenic mutation may be present in a different gene lying centromeric to the –16C>T substitution in the *puratrophin*-1 gene. Moreover, other patients sharing the common haplotype centromeric to the substitution in the *puratrophin*-1 gene without the substitution might exist.

In this study, we re-examined the haplotype of families showing ataxia in order to clarify a common genomic region shared in all 16q-ADCA patients. Because slippage mutation might cause minor deviations in repeat size for microsatellite markers (Ikeda et al. 2004), single nucleotide polymorphisms (SNPs) detected by ourselves on the disease chromosome were used in the analysis to confirm recombinant regions that are not conserved among families.

Materials and methods

Haplotype analysis

DNA samples from patients showing ataxia referred to our department were examined. After informed consent was obtained, genomic DNA was extracted from peripheral blood lymphocytes or lymphoblastoid cell lines by the use of methods described elsewhere (Ishikawa et al. 1997). All families were excluded for SCA1, SCA2, SCA3/ MJD, SCA6, SCA7, SCA8, SCA12, SCA14, SCA17, and DRPLA by testing for mutations in the disease genes.

Firstly, common haplotypes of the 16q-ADCA families with the –16C>T substitution in the *puratrophin*-1 gene were analyzed. Genotypes were determined for 19 microsatellite markers (D16S3043, D16S3031, D16S3019, CTATT01, TAGA02, GGAA05, D16S397, GGAA10, GATA01, D16S421, TA001, GA001, 17 msm, D16S3107, GGAA01, CTTT01, GT01, D16S3095, D16S512) in 16q22.1 by the use of methods described elsewhere (Ishikawa et al. 2005). Compared to our previous study (Ishikawa et al. 2005), several new markers with high specificity to the 16q-ADCA chromosome were added and the region analyzed was expanded to beyond the previous critical region spanning GATA01 and 17 msm (Ishikawa et al. 2005) in order to determine the maximum genomic region conserved in all of the affected individuals from all of the families. Although the phase of the markers were not confidently determined in families that have only a few examined members, the possibility that they carried the haplotype was indicated in those cases.

Secondly, haplotypes of families without the –16C>T substitution in the *puratrophin*-1 gene were also analyzed to see if they had the common haplotype centromeric to the substitution in the *puratrophin*-1 gene. Their genotypes were determined for 14 markers (D16S3043, D16S3019, CTATT01, TAGA02, GGAA05, D16S397, D16S3086, GATA01, GA001, 17 msm, CTTT01, GT01, D16S3095, D16S512), which are relatively highly specific to the common haplotype in 16q-ADCA.

Single nucleotide polymorphisms

We searched for single nucleotide polymorphisms (SNPs) on the disease chromosome by ourselves because most of

Table 1 The haplotype analysis of 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA) families with the -16CT substitution of the *puratrophin*-1 gene. The gray squares indicate that the alleles are one repeat-unit different fromthe common allele of 16q-ADCA and the black squares indicate alleles with two or more repeat-unitdifferences. One repeat-unit difference was seen for markers D16S397, GGAA10,GATA01, and TA001, close to the *puratrophin*-1 gene in several families, and greaterrepeat-units differences were observed for GGAA05 and other centromeric markers. Similarly, greater repeat-units differences were observed for 17msm and markers lyingtelomeric to 17msm. n.e.=not examined

Marker	most common haplotype	family No. / frequency in control (%)	P2	P4	P14	T2	T3	T4	T5	T6	T7	T12	T15	T19	T21	T25	T26	T28	T30	T37	T42	T43	T44	T46
D16S3043	1	25.0	1	1/6	**7**	**5**	**8**	1	1/8	1	1/5	**5**	1/7	n.e.	1/8	n.e.	n.e.	1	n.e.	1	n.e.	1/5	1/5	1
D16S3031	9	68.1	9	9	9	9	9	9	10	**1**	10	9	9/10	9	9	9	9	1/9	9	9	9	9	9	9
D16S3019	4	41.4	4	4	4	4	4	4/5	3/4	3/4	3/4	1/4	4	n.e.	4	n.e.	n.e.	4/7	3/4	2/4	n.e.	3/4	3/4	**1**
CTATT01	1	32.4	1	2/4	1	1	1	1/4	1	1	1	1	1/3	n.e.	1/3	n.e.	n.e.	1/3	n.e.	1	n.e.	1/2	1	0/3
TAGA02	4	10.3	4	**6**	4	4	4	4/6	4/6	4/5	4	2/4	4/5	n.e.	4/5	n.e.	n.e.	4/5	4	3/4	n.e.	5/6	4/5	**2/6**
GGAA05	1	1.4	1	**6**	1	1	**5**	1	1	1	2	1	2/4	1/3	1/5	**5**	1/2	1/7	1/5	1/3	1/3	1/5	2/4	**3/6**
D16S397	1	47.1	n.e.	1/2	1	1	1	0/4	1/2	1/3	1/3	2/3	1	n.e.	1	n.e.	n.e.	1/4	-3/0	-3/1	n.e.	-3/1	1	-3/1
GGAA10	3	13.2	3	3	3	3	4	3/5	3	3	3	3	3	3/6	2/3	2/4	3/7	3/5	3/8	4/6	3	3/6	3/5	3/7
GATA01	2	44.1	2	2/3	2	2	2	3	3	3	2	2	1/2	2/3	1/3	3/4	1/3	1/3	1	2	3	1/3	2/3	2/3
D16S421	3	75.7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3/4	3	3	3
puratrophin-1(C/T)	T	0.0	T	T	T	T	T	T	T	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C
TA001	1	23.8	1	1	1	1	1	1	1	1	1	1	1	1/9	1/7	2/8	1/6	1/10	1/9	1/9	1	1/9	1/9	1/5
GA001	4	0.1	4	4	4	4	4	4	4	4/7	4/7	4	4	1/4	4/8	4/5	4/7	4/7	4/6	4/11	4/5	4/7	4/5	4/7
17msm	2	8.3	2	2	2	2	2	2	2	2	2	**5**	2	2/5	2/5	2/4	2/4	2/4	2/4	2/5	2/4	2/4	**4/6**	2/4
D16S3107	7	13.9	7	7	7	7	7	7	6	7	7	7	**5**	6	5/6	3/7	6/10	6	7	5/7	3/7	6/7	4/7	6/7
GGAA01	6	18.8	6	6	6	6	6	6	6	7	6	6	1/6	3/6	2/6	2/6	4/6	6/7	6	6	6	6/7	6/7	5/6
CTTT01	8	28.2	8	8	8	8	9/10	8	**5**	9	8	**10**	8	9/10	3/9	9/10	8/10	8	8/11	6/9	8/15	9/10	1/7	3/8
GT01	6	15.8	6	6	6	6	6	6	7	6	6	**4**	6	2/6	1/6	4/6	4/6	2/6	5/6	4/6	6	3/6	**3/4**	4/6
D16S3095	1	9.7	2	**3**	1	**0**	1	1	2	**3**	2	1/2	1	1	1/2	1/2	1/2	1/3	1	1	1/2	1/3	2	1
D16S512	1	32.3	n.e.	2/4	1	**4**	1/5	**4**	2/4	1/5	1/5	1/5	**4**	n.e.	**4/5**	n.e.	n.e.	**5**	1	1/2	n.e.	2/4	**4**	**4/5**

the SNPs obtained from public databases were not present on the disease allele or did not have enough specificity to the disease chromosome. SNPs were revealed by direct sequencing of the genomic DNA from a homozygous patient who carries the common haplotypes between D16S3031 and GT01 in both of the chromosomes. Primers were designed to amplify about 800 bp from genomic DNA (primer sequences are available on request), and polymerase chain reaction (PCR) and sequencing were performed with the same methods as previously described (Ishikawa et al. 2005). Comparing the sequenced data and the annotated databases with use of DNASIS (Hitachi) software revealed many SNPs. With the sequenced data of the control genomic DNA, SNPs with high specificity to the 16q-ADCAs were chosen. With these SNPs, 16q-ADCA families were analyzed to reveal the borders of the maximally conserved genomic region.

Results

Haplotype analysis of 16q-ADCA with the –16C>T substitution in the *puratrophin*-1 gene

One hundred and twenty-five patients from 64 families were diagnosed as 16q-ADCA based on the clinical features and the presence of the –16C>T substitution in the *puratrophin*-1 gene. The families included 52 families that we had previously reported (Ishikawa et al. 2005) and 12 new families that had not been reported elsewhere. They all share similar haplotypes around the *puratrophin*-1 gene. The most common haplotype among these families are shown in the left column in Table 1. Twenty-two families out of the 64 families showed different alleles at least for one of the DNA markers as shown in Table 1. The remaining 42 families, which are not listed in Table 1, harbored or had the possibility to harbor the common haplotype.

There was one repeat-unit difference from the common alleles for D16S397, GGAA10, GATA01, and TA001 close to the *puratrophin*-1 gene in 13 out of 22 families. For centromeric DNA markers from the *puratrophin*-1 gene, such as GGAA05, TAGA02, D16S3031, and D16S3043, eight families (P4, P14, T2, T3, T6, T12, T25, T46) harbored alleles with greater differences in repeat number (more than two repeat-units). Furthermore, families P4 and T46 carried different alleles in three consecutive markers, GGAA05, TAGA02, and CTATT01.

Similarly, for telomeric DNA markers such as 17 msm, D16S3107, CTTT01, and GT01, greater differences were seen in three families (T12, T15, T44). Especially, families T12 and T44 harbored different alleles for markers 17 msm, CTTT01, and GT01, which were highly specific to the common haplotype.

The presence of large differences in repeat number and successively different alleles would indicate that the families were sharing the common chromosomal region,

inherited from a founder, between markers GGAA05 and 17 msm.

Haplotype analysis of families without identifiable genetic mutations

Twenty-three patients from 22 families presenting pure cerebellar ataxia did not carry identifiable genetic mutations. Nine families showed autosomal dominant inheritance, and the other families had no apparent family history. Their haplotypes are shown in Table 2. Although no family carried entirely identical alleles to the common haplotype consecutively for the markers telomeric to the *puratrophin*-1 gene, one family (U09) harbored the identical alleles for the markers between D16S3043 and GATA01 centromeric to the *puratrophin*-1 gene. It suggested the possibility that the U09 family have the common haplotype of 16q-ADCA in the region centromeric to the −16C>T substitution in the *puratrophin*-1 gene.

Haplotype analysis with SNPs

Four markers, GGAA05, D16S397, GGAA10, and GATA01 centromeric to the *puratrophin*-1 gene, showing different alleles in Table 1 suggested that ancestral chromosomal recombination might have occurred around the markers. Family U09 and the family reported by Ohata et al. 2006) also suggested ancestral chromosomal recombination around the substitution in the *puratrophin*-1 gene. Therefore, we searched the SNPs around these four markers and the *puratrophin*-1 gene. Five SNPs were identified around the marker GGAA05, one SNP around D16S397, four SNPs around GGAA10, one SNP around GATA01, and two SNPs around the *puratrophin*-1 gene (Table 3). SNP05 and SNP06 showed high specificity to the disease chromosome because they were absent in 200 control chromosomes.

Eighteen families showed different alleles for GGAA05, D16S397, GGAA10, or GATA01 (Table 1). Among them, sufficient amounts of DNA samples were not available in four families (T25, T26, T30, T42). The remaining 14 families were analyzed as shown in Table 4. While 13 out of the 14 families carried all of the same SNPs, family T46 did not carry SNP01, SNP02, SNP03, and SNP04. This confirmed that the genomic region between SNP01 and SNP04 of family T46 was a recombinant region, which was not conserved in all families.

These SNPs were also analyzed for the U09 family suspected of having the common haplotype of 16q-ADCA (Table 4). The family had all 13 SNPs, including SNP05 and SNP06, which are highly specific to the disease chromosome. This strongly suggested that family U09 shared the 16q-ADCA common haplotype centromeric to the −16C>T substitution in the *puratrophin*-1 gene.

Discussion

16q-ADCA is one of the most common ataxic diseases in Japan. We previously showed that 52 families shared the common haplotype in the genomic 400-kb region between the markers GATA01 and 17 msm by analysis with

Table 2 The haplotype analysis of families without identifiable genetic mutation. The black squares indicate that the families carry the identical alleles to the common alleles of 16q-ADCA, and the gray squares indicate alleles with one repeat-unit difference. Only family U09 harbored the identical alleles consecutively for the markers from D16S3043 to GATA01, suggesting that this family may harbor the common haplotype of 16q-ADCA. n.c.=notclear. A.D.=autosomal dominant inheritance was suspected

		Family No.	U01	U02	U03	U04	U05	U06	U07	U08	U09	U10	U11	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21	U22
		Family history	n.c.	n.c.	n.c.	A.D.	n.c.	n.c.	n.c.	A.D.	n.c.	n.c.	A.D.	A.D.	A.D.	n.c.	A.D.	A.D.	A.D.	A.D.	n.c.	n.c.	n.c.	n.c.
Marker	most common haplotype	frequency in control (%)																						
D16S3043	1	25.0	1/6	4/8	1/2	1/7	1	4/6	5	5	1/7	1/7	4/5	4/5	1/6	1/5	5	1/6	5/7	1/6	1	1	1/5	1/8
D16S3019	4	41.4		1/4	4	1	3/4	5	3/4	4	4	3/4	1	2	4	4/5	4/5	3/4	1	2	1	1	1/3	1/2
CTATT01	1	32.4	3/4	1/4	1/3	3	2/4	1/2	3	3	1/4	1/3	1/3	1/3	4	1	1/3	2/5	1	2/3	1/3	1/2	1	1/3
TAGA02	4	10.3	3/5	6	2/4	4/5	5/6	4/6	5/6	5/6	4/5	5	5	6/7	5	2/6	3/5	4/5	6	4/5	4/6	3/6	2/6	5/6
GGAA05	1	1.4	3	4/5	4/5	4	5	3/4	4/6	4	1/3	3	2/4	4	4	5	4/5	2/6	4	4/5	4/6	5/6	4/6	4/5
D16S397	1	47.1	-1/1	1/3	2/3	4/6	4	1	3	4	1	3	1/4	1	1	1/2	1/4	3/5	1/4	2/6	1	-3/3	1/3	-3/6
D16S3086	2	65.7	2	2/3	3/4	3/4	3	2	3	3	2	3	2/4	2	2	2	2/3	2/3				3	2/3	3/4
GATA01	2	44.1	2	1/2	1/3	2/4	3	3	1/3	3	1/2	2/3	2	2/3	2	2/3	2/3	2	1	0/2	3/4	2	1	0/2
puratrophin-1 (C/T)	T	0.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
GA001	4	0.1	1/8	6/8	8	8/9	8	7/11	8/9	1	5/7	7/10	6/7	5/8	5	5/8	5/9	1/7	6/7	5/7	6/9	6/8	7/9	7/8
17msm	2	8.3	3/4	4	4/5	4/5	5	2/6	5	2	5	3/4	4	3/4	4	1/3	2/4	7	5	2/6	4	2/4	4/5	2/5
CTTT01	8	28.2	5/7	8/10	6/10	6/9	4/5	7/10	5/6	9	5/10	6/7	9	7/9	5/6	7/8	7/10	5/11	5/7	7/10	8/10	4/7	6/8	7/9
GT01	6	15.8	2/4	4/6	2/6	2/4	2/5	1/2	2/3	7	2/4	2/6	1/4	4/6	3/4	4	2/6	3/4	4/7	2/6	3/6	4/6	5/8	2
D16S3095	1	9.7	2	1/3	2/3	2/5	1/3	2/3	2/4	6	2	2/3	2/4	1/2	3	2/3	1/2	1/3	3/4	3/4	1/2	2/6	2/4	2/4
D16S512	1	32.3	2/4	1/5	4/5	2/4	2/4	4	2/4	1	2	2/4	1/4	4/5	4	2/4	4	2/3	1/4	2	4/5	4/5	4/5	4

Table 3 Single nucleotide polymorphisms (SNPs) on the disease chromosome of 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA). We identified thirteen SNPs by ourselves. SNP05 and SNP06 were absent in control chromosomes (*n*=200) and are thought to be highly specific to the disease chromosome

SNP/marker		Position on Chr 16	SNP change on 16q-ADCA	Frequency in control (%)
	GGAA05	64,938,933		
SNP01		64,972,150	A → G	27.8
SNP02		64,977,170	A → C	22.2
SNP03		64,977,733	T → C	30.0
SNP04		64,982,678	C → T	27.8
SNP05		65,049,292	G → A	0.0
.	D16S397	65,295,770		
SNP06		65,337,827	A → G	0.0
SNP07		65,449,825	C → T	56.3
SNP08		65,451,833	T → A	45.5
	GGAA10	65,452,426		
SNP09		65,457,741	T → A	42.4
SNP10		65,458,302	T → C	45.5
SNP11		65,669,454	T → C	30.3
	GATA01	65,700,022		
SNP12		65,771,917	G → A	18.2
SNP13		65,793,152	C → T	8.7
puratrophin-1 (C/T)		65,871,434	C → T	0.0

microsatellite markers. Within this region, we had found that the single nucleotide −16C>T substitution in the *puratrophin*-1 gene was strongly associated with the disease (Ishikawa et al. 2005). Since then, a number of patients with the substitution and the common haplotype were reported in various areas of Japan. However, a report of the one exceptional patient without the substitution in the family in which all other affected subjects carried the substitution (Ohata et al. 2006) raised the possibility that a true pathogenic mutation may be present in a different gene. This exceptional patient indicated that the mutation might be lying centromeric to the substitution in the *puratrophin*-1 gene, where the patient shared the common haplotype with other affected individuals in the family.

Here, we re-examined the 16q-ADCA families with the −16C>T substitution in the *puratrophin*-1 gene with microsatellite markers and found four possible centromeric borders of the disease locus (GATA01, D16S397, GGAA10, GGAA05), based on the difference of alleles. We searched for informative SNPs around the markers capable of distinguishing the chromosomes derived from a founder and analyzed haplotypes with the SNPs. Because all of the examined families carried SNPs around the markers GATA01, D16S397, and GGAA10, ancestral chromosomal recombination around the markers was not confirmed. The differences in alleles for these markers was only one repeat-unit, suggesting that the allele differences

Table 4 The haplotype analysis with single nucleotide polymorphisms (SNPs). Fourteen familiesof 16q-ADCA with different alleles for microsatellite markers and family U09 are shown.The gray squares indicate that the family carried the SNPs common to 16q-ADCA. Family T46 did not carry the common SNPs from SNP01 to SNP04. This is consistent with the findingon microsatellite markers (Table 1), further suggesting that the centromeric border of thedisease locus is SNP04. Family U09 carried all of the 13 SNPs. This would also supportthe theory that family U09 shares the 16q-ADCA common haplotype centromeric to the substitution inthe *puratrophin*-1 gene

SNP	SNP change on 16q-ADCA	frequency in control (%)	family No.														
			P4	T3	T4	T5	T6	T7	T12	T15	T21	T28	T37	T43	T44	T46	U09
SNP01	A → G	27.8	G/A	G/A	G/A	G	G	G/A	G	G/A	G/A	G/A	G	G/A	G	A	G/A
SNP02	A → C	22.2	C/A	C/A	C/A	C	C	C/A	C	C/A	C/A	C/A	C	C/A	C/A	A	C/A
SNP03	T → C	30.0	C/T	C/T	C/T	C	C	C/T	C	C/T	C/T	C/T	C	C/T	C	T	C/T
SNP04	C → T	27.8	T/C	T	T/C	T	T	T/C	T	T/C	T/C	T	T	T/C	T	C	C/T
SNP05	G → A	0.0	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G
SNP06	A → G	0.0	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
SNP07	C → T	56.3	T	T/C	T/C	T	T	T/C	T/C	T	T	T/C	T/C	T/C	T	T/C	T/C
SNP08	T → A	45.5	A	A/T	A/T	A	A	A/T	A/T	A	A	A/T	A/T	A/T	A	A/T	A
SNP09	T → A	42.4	A	A/T	A/T	A	A	A/T	A/T	A	A	A/T	A/T	A/T	A	A/T	A
SNP10	T → C	45.5	C	C/T	C/T	C	C	C/T	C/T	C	C	C/T	C/T	C/T	C/T	C/T	C
SNP11	T → C	30.3	C	C/T	C/T	C/T	C	C/T	C	C	C/T	C/T	C/T	C/T	C	C/T	C/T
SNP12	G → A	18.2	A	A/G	A/G	A/G	A/G	A/G	A/G	A	A/G	A/G	A/G	A/G	A	A/G	A/G
SNP13	C → T	8.7	T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	T	C/T	C/T
puratrophin-1(C/T)	C → T	0.0	T	T	T	T	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	C

in GATA01, D16S397, and GGAA10 might have resulted not from recombination events, but from the microsatellite slippage mutation (Ikeda et al. 2004). On the other hand, four families (P4, T3, T25, T46) showed great allele differences in GGAA05 and one family (T46) did not carry four SNPs, confirming that family T46 did not share the genomic region centromeric to GGAA05 with the other 16q-ADCA families. This strongly indicates that the centromeric border of the disease locus of 16q-ADCA could be placed at SNP04.

The U09 family had the identical alleles for all markers and SNPs in the region centromeric to the −16C>T substitution in the *puratrophin*-1 gene. It is impossible to conclude that the family has the common haplotype of 16q-ADCA because only one examined family member was available for the present genetic analysis. However, carrying the rare alleles for GGAA05 and infrequent SNPs, both highly specific to the disease chromosome, strongly suggests that the U09 family shares a part of the 16q-ADCA common haplotype. The patient in the U09 family developed pure cerebellar ataxia later in life without apparent family history. Because 16q-ADCA patients were found among sporadic cases (Ouyang et al. 2006), these clinical features of the U09 family are consistent with those of 16q-ADCA. Importantly, this family had not been reported previously and, therefore, would be the second case of 16q-ADCA without the substitution in the *puratrophin*-1 gene following the family reported by Ohata et al. (2006). These cases indicate that the telomeric end of the disease locus could be placed at the −16C>T substitution in the *puratrophin*-1 gene.

Haplotype analysis of a number of 16q-ADCA families with microsatellite markers and SNPs in this study suggests

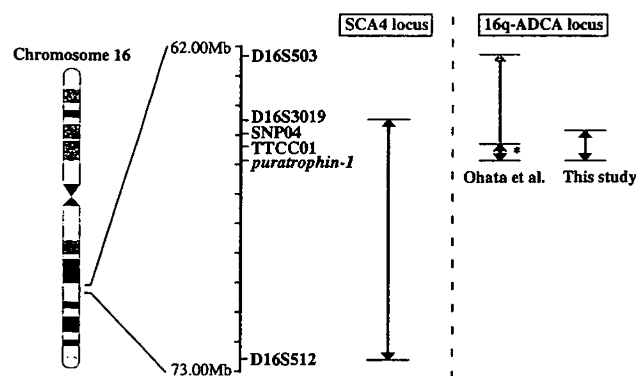

Fig. 1 A summary of critical intervals for 16q-ADCA and SCA4. Our study could define the disease locus of 16q-ADCA to a 900-kb genomic region between SNP04 and the −16C>T substitution in the *puratrophin*-1 gene. This region is completely inside the candidate locus of SCA4 (Flanigan et al. 1996).The haplotype region (*asterisk*) between TTCC01 and the *puratrophin*-1 gene shown by Ohata et al. (2006) is also shown, together with an alternative critical region between D16S503 and the *puratrophin*-1 gene (see text for details)

that the gene locus of 16q-ADCA could be re-assigned to a 900-kb genomic region between SNP04 and the substitution in the *puratrophin*-1 gene (Fig. 1). This region partly overlaps with, but is not the same as, the candidate region previously set by Ohata et al. (2006). They showed that three large 16q-ADCA families shared a common haplotype between D16S3086 and D16S412, and suggested the possibility that real pathogenic mutation would exist in the region between TTCC01 and the −16C>T substitution in the *puratrophin*-1 gene. However, the allele difference for TTCC01 in their families was only one repeat-unit, and all of their patients shared identical allele for TAGA02, lying centromeric to TTCC01. Since the possibility of slippage mutation remains as an explanation for the allele difference seen in TTCC01, as we observed for GATA01, D16S397, and GGAA10, it would be cautious to place the centromeric border at the marker TTCC01. Given that the allele differences in TTCC01 is due to slippage mutation, the centromeric border in their families would be alternatively set at D16S503, since an obligate recombination was seen between D16S503 and TAGA02. It would be, thus, important to analyze GGAA05 and specific SNPs in their families to see to what extent their patients harbor conserved haplotypes.

Although we found a patient without the −16C>T substitution in the *puratrophin*-1 gene, the substitution was present in all patients except the one in the U09 family (i.e., 125/126=99.2% sensitivity; 100% specificity) and, thus, the *puratrophin*-1 genetic change still remains to be a useful marker. Molecular diagnosis with multiple microsatellite markers and SNPs will help to identify 16q-ADCA patients more accurately. Through the present study, we showed that the truly pathogenic mutation would lie in a 900-kb genomic region between SNP04 and the −16C>T substitution in the *puratrophin*-1 gene. Further investigations for finding a genetic mutation within the critical region are needed to elucidate the molecular pathogenesis of 16q-ADCA.

References

Cagnoli C, Mariotti C, Taroni F, Seri M, Brussino A, Michielotto C, Grisoli M, Di Bella D, Migone N, Gellera C, Di Donato S, Brusco A (2006) SCA28, a novel form of autosomal dominant cerebellar ataxia on chromosome 18p11.22-q11.2. Brain 129:235–242

Chen DH, Brkanac Z, Verlinde CL, Tan XJ, Bylenok L, Nochlin D,Matsushita M, Lipe H, Wolff J, Fernandez M, Cimino PJ, Bird TD,Raskind WH (2003) Missense mutations in the regulatory domain of PKC gamma: a new mechanism for dominant nonepisodic cerebellar ataxia. AmJ Hum Genet 72:839–849

Flanigan K, Gardner K, Alderson K, Galster B, Otterud B, Leppert MF, Kaplan C, Ptacek LJ (1996) Autosomal dominant spinocerebellar ataxia with sensory axonal neuropathy (SCA4): clinical description and genetic localization to chromosome 16q22.1. Am J Hum Genet 59:392–399

Harding AE (1982) The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of the "Drew family of Walworth." Brain 105:1–28

Hellenbroich Y, Pawlack H, Rub U, Schwinger E, Zuhlke Ch (2005) Spinocerebellar ataxia type 4. Investigation of 34 candidate genes. J Neurol 252:1472–1475

Holmes SE, O'Hearn EE, McInnis MG, Gorelick-Feldman DA, Kleiderlein JJ, Callahan C, Kwak NG, Ingersoll-Ashworth RG, Sherr M, Sumner AJ, Sharp AH, Ananth U, Seltzer WK, Boss MA, Vieria-Saecker A-M, Epplen JT, Riess O, Ross CA, Margolis RL (1999) Expansion of a novel CAG trinucleotide repeat in the 5′ region of PPP2R2B is associated with SCA12. Nat Genet 23:391–392

Ikeda Y, Dalton JC, Moseley ML, Gardner KL, Bird TD, Ashizawa T, Seltzer WK, Pandolfo M, Milunsky A, Potter NT, Shoji M, Vincent JB, Day JW, Ranum LP (2004) Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. Am J Hum Genet 75:3–16

Ikeda Y, Dick KA, Weatherspoon MR, Gincel D, Armbrust KR, Dalton JC, Stevanin G, Durr A, Zuhlke C, Burk K, Clark HB, Brice A, Rothstein JD, Schut LJ, Day JW, Ranum LP (2006) Spectrin mutations cause spinocerebellar ataxia type 5. Nat Genet 38:184–190

Ishikawa K, Tanaka H, Saito M, Ohkoshi N, Fujita T, Yoshizawa K,Ikeuchi T, Watanabe M, Hayashi A, Takiyama Y, Nishizawa M, Nakano I,Matsubayashi K, Miwa M, Shoji S, Kanazawa I, Tsuji S, Mizusawa H(1997) Japanese families with autosomal dominant pure cerebellar ataxia map to chromosome 19p13.1-p13.2 and are strongly associated with mild CAG expansions in the spinocerebellar ataxia type 6 gene in chromosome 19p13.1. Am J Hum Genet 61:336–346

Ishikawa K, Toru S, Tsunemi T, Li M, Kobayashi K, Yokota T, Amino T, Owada K, Fujigasaki H, Sakamoto M, Tomimitsu H, Takashima M, Kumagai J, Noguchi Y, Kawashima Y, Ohkoshi N, Ishida G, Gomyoda M, Yoshida M, Hashizume Y, Saito Y, Murayama S, Yamanouchi H, Mizutani T, Kondo I, Toda T, Mizusawa H (2005) An autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 is associated with a single-nucleotide substitution in the 5′ untranslated region of the gene encoding a protein with spectrin repeat and Rho guanine nucleotide exchange-factor domains. Am J Hum Genet 77:280–296

Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, Ranum LP (1999) An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat Genet 21:379–384

Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY, Ashizawa T (2000) Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. Nat Genet 26:191–194

Nagaoka U, Takashima M, Ishikawa K, Yoshizawa K, Yoshizawa T, Ishikawa M, Yamawaki T, Shoji S, Mizusawa H (2000) A gene on SCA4 locus causes dominantly inherited pure cerebellar ataxia. Neurology 54:1971–1975

Ohata T, Yoshida K, Sakai H, Hamanoue H, Mizuguchi T, Shimizu Y, Okano T, Takada F, Ishikawa K, Mizusawa H, Yoshiura K, Fukushima Y, Ikeda S, Matsumoto N (2006) A −16C>T substitution in the 5′ UTR of the puratrophin-1 gene is prevalent in autosomal dominant cerebellar ataxia in Nagano. J Hum Genet 51:461–466

Onodera Y, Aoki M, Mizuno H, Warita H, Shiga Y, Itoyama Y (2006) Clinical features of chromosome 16q22.1 linked autosomal dominant cerebellar ataxia in Japanese. Neurology 67:1300–1302

Ouyang Y, Sakoe K, Shimazaki H, Namekawa M, Ogawa T, Ando Y, Kawakami T, Kaneko J, Hasegawa Y, Yoshizawa K, Amino T, Ishikawa K, Mizusawa H, Nakano I, Takiyama Y (2006) 16q-linked autosomal dominant cerebellar ataxia: a clinical and genetic study. J Neurol Sci 247:180–186

Ross CA, Poirier MA (2004) Protein aggregation and neurodegenerative disease. Nat Med 10 Suppl:S10–S17

Sasaki H, Yabe I, Tashiro K (2003) The hereditary spinocerebellar ataxias in Japan. Cytogenet Genome Res 100:198–205

Schöls L, Bauer P, Schmidt T, Schulte T, Riess O (2004) Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 3:291–304

van Swieten JC, Brusse E, de Graaf BM, Krieger E, van de Graaf R, deKoning I, Maat-Kievit A, Leegwater P, Dooijes D, Oostra BA, Heutink P(2003) A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebellar ataxia. Am J Hum Genet72:191–199

Takano H, Cancel G, Ikeuchi T, Lorenzetti D, Mawad R, Stevanin G, Didierjean O, Durr A, Oyake M, Shimohata T, Sasaki R, Koide R, Igarashi S, Hayashi S, Takiyama Y, Nishizawa M, Tanaka H, Zoghbi H, Brice A, Tsuji S (1998) Close associations between prevalences of dominantly inherited spinocerebellar ataxias with CAG-repeat expansions and frequencies of large normal CAG alleles in Japanese and Caucasian populations. Am J Hum Genet 63:1060–1066

Takashima M, Ishikawa K, Nagaoka U, Shoji S, Mizusawa H (2001) A linkage disequilibrium at the candidate gene locus for 16q-linked autosomal dominant cerebellar ataxia type III in Japan. J Hum Genet 46:167–171

Waters MF, Minassian NA, Stevanin G, Figueroa KP, Bannister JP, Nolte D, Mock AF, Evidente VG, Fee DB, Muller U, Durr A, Brice A, Papazian DM, Pulst SM (2006) Mutations in voltage-gated potassium channel KCNC3 cause degenerative and developmental central nervous system phenotypes. Nat Genet 38:447–451

Wieczorek S, Arning L, Alheite I, Epplen JT (2006) Mutations of the puratrophin-1 (PLEKHG4) gene on chromosome 16q22.1 are not a common genetic cause of cerebellar ataxia in a European population. J Hum Genet 51: 363–367

Yu GY, Howell MJ, Roller MJ, Xie TD, Gomez CM (2005) Spinocerebellar ataxia type 26 maps to chromosome 19p13.3 adjacent to SCA6. Ann Neurol 57:349–354

短　報

嚥下造影検査が重症筋無力症増悪の評価に有効であった1例

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大矢　　寧　　小川　雅文　　村田　美穂

要旨：重症筋無力症（MG）の嚥下障害の評価に，固形物の嚥下造影検査（VF）による塩化エドロフォニウム（EC）試験が有用であった64歳の女性例を報告した．62歳時に，頸部筋力低下，複視，左眼瞼下垂でMGを発症し，拡大胸腺摘除をおこなった．64歳時，プレドニゾロン（PSL）の内服で症状は改善していたが，嚥下困難感と開鼻声が出現した．EC試験では嚥下困難感の自覚的な改善に乏しく，開鼻声，僧帽筋の神経反復刺激試験も改善しなかった．VFではEC静注後の固形物の咀嚼嚥下が改善し，MGの増悪と診断できた．PSL増量後，嚥下困難感は消失し，VFで嚥下機能の改善が確認された．

（臨床神経，47：669—671, 2007）
Key words：重症筋無力症，嚥下障害，嚥下造影検査，エドロフォニウム試験，咀嚼嚥下

はじめに

重症筋無力症（myasthenia gravis：MG）患者の嚥下性肺炎の合併はクリーゼの危険因子とされるため嚥下障害の評価は重要である[1]．嚥下機能の評価には塩化エドロフォニウム（edrophonium chloride：EC）静注前後の嚥下造影検査（videofluorography：VF）の比較が有用であるが，VFで使用する造影剤加模擬食品についての検討は少ない[2)~4)]．EC静注前後の固形物のVFがMGの嚥下機能の評価に有用であった症例を報告する．

症　例

患者：64歳　女性．

主訴：飲み込みづらい．

既往歴・生活歴・家族歴：いずれも特記事項なし．

現病歴：62歳時，臥位での頭部挙上困難，左眼瞼下垂，複視が出現した．血清抗アセチルコリン受容体抗体（抗AChR抗体）63nmol/L，左僧帽筋の神経反復刺激試験は減衰率25%で，MGと診断された．拡大胸腺摘除術を受けたところ，胸腺過形成であった．ピリドスチグミン180mgを内服し，寛解した．63歳時，呼吸不全，左眼瞼下垂，複視が出現し，3日間，非侵襲的陽圧換気で管理された．経口プレドニゾロン（PSL）を開始し，70mgまで漸増して症状は改善した．その後，ピリドスチグミン120mg連日とPSL 20mg隔日の内服で寛解した．64歳時，頸部の筋力低下が増悪し，左眼瞼下垂，複視が出現した．PSL 20mg隔日内服から25mg連日内服に増量し，ピリドスチグミン120mgを維持し，症状は軽快した．寛解してその半年後，食事で喉にひっかかる感じやむせこみ，開鼻声が出現したため，入院した．

現症：身長158cm，体重68kg，血圧168/88mmHg，脈拍82/分整，呼吸回数17回/分であった．意識障害や心気傾向はなかった．開鼻声，複視，左眼瞼下垂をみとめ，これらの症状は夕方になると増悪した．通常の食事は経口摂取可能で，咀嚼中の疲労の訴えはなかったが，喉に詰まる感じを訴えた．液体の嚥下ではときにむせ込んだ．咽頭反射，軟口蓋反射，舌運動は正常であった．筋力は寛解時と変化なく，徒手筋力テストで頸部前屈筋2，肩外転筋は右4，左2，肩挙上筋は両側4で，その他の筋力は正常であった．

検査：血算・血液生化学は正常で，CK 64 IU/L，抗AChR抗体25nmol/Lと寛解時と著変なかった．座位での動脈血液ガスは室内気でpH 7.42，$PaCO_2$ 43.5mmHg，PaO_2 75.2 mmHg，SaO_2 94%，肺活量は1.75L（予測値の71.6%），ピークフローは5.47L/sであった．胸部X線写真，胸部CTでは肺野と縦隔に異常なかった．頭部MRIは正常であった．EC 10mg静注後，開鼻声，複視，眼瞼下垂，食物の飲み込みづらさは改善せず，僧帽筋での反復刺激試験でも減衰率56%でEC静注前と変化なかった．

VF：「嚥下造影の標準的手順」[5]に準拠し，書面による同意をえて検査した．EC 10mg静注前後でバリウム水溶液10mlを検者の合図で嚥下する命令嚥下とバリウム加コンビーフ8gを自由に咀嚼し，任意のタイミングで嚥下する咀嚼嚥下を30フレーム/秒で記録した．照射時間を減らすためプラセボの評価はしなかった．それぞれの嚥下について，1)嚥下回数，2)咽頭での残留の有無，3)バリウムが下顎下縁を越え輪状咽

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（受付日：2007年3月14日）

Table 1 Comparison of videofluorography results before and after edrophonium chloride (EC) injection

	Liquid (10 ml barium)			Solid food (8 g of corned beef hash)		
	At the time of admission		3 months after admission	At the time of admission		3 months after admission
	Before EC	After EC		Before EC	After EC	
1) Number of swallows	3	1	1	5	1	2
2) Residue in VAL and	++	+	−	++	−	+
in piriform fossa	+	+	−	+	−	−
3) Pharyngeal transit duration (s)	0.84	0.66	0.59	2.86	1.99	2.17
4) Aspiration	None	None	None	None	None	None

EC: edrophonium chloride. VAL: valleculae. The initiation of swallowing is defined as the starting point of the sudden and rapid superior and anterior motion of the hyoid bone. A3 point scale was used; − corresponds to no residue; +, to a coating of residue (a line of barium on a structure); and ++, to pooling of barium. There was no evidence of aspiration in any of the recordings. The first videofluorography was performed at the time of admission. After the EC injection, the number of swallows decreased, and the residue disappeared, particularly with solid food. The patient was treated with 70 mg of PSL for 4 weeks, and the second videofluorography was performed 3 months after the first videofluorography. The number of swallows, the residue, and pharyngeal transit duration improved.

Fig. 1 Videofluorography

Videofluorography performed while consumption of 8 g of corned beef hash with barium. On the left: Before edrophonium chloride (EC) injection. The residue is prominent in the valleculae (large arrow) and the hypopharynx (small arrows). On the right: After 10 mg EC injection. No residue is observed in the valleculae and the piriform fossa following the ingestion of corned beef hash. Dysphagia was improved by EC injection. The ingestion of corned beef hash was useful in evaluating the swallowing disturbance. The round objects are 24 mm in diameter (lower right arrow).

頭筋を通過するまでの時間（pharyngeal transit duration：PTD）[6]，4）誤嚥の有無をコンピューターで解析した．

VF 結果：液体の命令嚥下では，EC 静注後，嚥下回数は 3 回から 1 回に減り，喉頭蓋谷の残留は軽度減少し，PTD は 0.18 秒短縮したが，鼻腔へのバリウム逆流と，梨状陥凹の残留は不変だった．咀嚼嚥下では，EC 静注後，嚥下回数は 5 回から 1 回に減り，PTD は 1.87 秒短縮したが，喉頭蓋谷と梨状陥凹の残留は消失した．いずれの検査でも，誤嚥はなかった（Table 1，Fig. 1）．EC 静注後，自覚症状の改善はなかった．

経過：PSL 70mg 連日を 4 週間内服後，嚥下困難感は消失した．VF では，液体の命令嚥下と咀嚼嚥下ともに，嚥下回数と咽頭の残留は減少し，PTD は短縮した（Table 1）．

考　察

寛解にみえた MG 患者が嚥下困難感を訴え，EC 試験では固形物の VF 所見が改善したことから MG による嚥下障害と診断した．食事中のむせ込みや咀嚼での疲労はなく，液体嚥下ではむせ込みはまれで，評価の指標にはならなかった．EC 10mg 静注後の嚥下においても他覚所見と自覚症状に乖離があり，MG では VF による客観的な評価が必要であることを示した．なお，複視や眼瞼下垂，開鼻声は EC 10mg では改善なく，MG では筋の部位によって EC に対する反応性がことなることが示唆された．

MGによる嚥下障害は，嚥下の反復で増悪する嚥下動作の遅延，咽頭での食物残留などを特徴とし，コリン作動性クリーゼによる嚥下障害との鑑別には，EC静注後の嚥下機能の評価が有用である[2)~4)]．EC試験と同時に固形物のVFをおこなった報告として，経口摂取可能なMG患者に20分間食事をさせた後にVFをおこない，EC静注後に喉頭蓋谷の食物の残留が消失した症例がある[7)]．本例では，バリウム加コンビーフを選択し，過剰な負荷をかけてVF中の誤嚥の危険性を高めることなく，客観的に嚥下機能の改善を評価できることを示した．評価には，嚥下回数，咽頭のバリウム残留，咽頭でのバリウム通過時間が有用であった．

一般に液体の命令嚥下では重力による受動的輸送の要素が強く，固形物の咀嚼嚥下では咽頭筋群の収縮によって食物を送り込む能動的輸送と重力の両方が関与している[8)]．MGによる嚥下障害では重力による受動的輸送は悪化せず，EC静注で舌筋や咽頭筋群による能動的輸送が改善すると考えられ，病態の評価には液体よりも固形物のVFが良いと考えた．また，固形物のVFは液体のVFよりも喉頭蓋谷や梨状陥凹の残留を確認しやすいため，ECの効果を検査室で視認しやすい点でも有用であると考えた．

嚥下障害があるMG患者のVFの適応には十分な検討が必要であるが，造影剤加模擬食品として固形物の選択が有用であった例を報告した．

本論文の要旨は第173回日本神経学会関東地方会（2005年6月）で発表した．

文　献

1) Colton-Hudson A, Koopman WJ, Moosa T, et al: A prospective assessment of the characteristics of dysphagia in myasthenia gravis. Dysphagia 2002; 17: 147—151
2) Viets HR: Myasthenia Gravis. Springfield, Illinois, 1961, pp 346—361
3) Murray JP: Deglutition in myasthenia gravis. Br J Radiol 1962; 35: 43—52
4) Higo R, Nito T, Tayama N: Videofluoroscopic assessment of swallowing function in patients with myasthenia gravis. J Neurol Sci 2005; 231: 45—48
5) 日本摂食・嚥下リハビリテーション学会医療検討委員会：嚥下造影の標準的検査法（詳細版）．日摂食嚥下リハ会誌　2004；8：71—86
6) Robbins JA, Logemann JA, Kirshner HS: Swallowing and speech production in Parkinson's disease. Ann Neurol 1986; 19: 283—287
7) Schwartz DC, Waclawik AJ, Ringwala SN, et al: Clinical utility of videofluorography with concomitant Tensilon administration in the diagnosis of bulbar myasthenia gravis. Dig Dis and Sci 2005; 50: 858—861
8) 松尾浩一郎，才藤栄一，武田斉子ら：咀嚼および重力が嚥下反射開始時の食塊の位置に及ぼす影響．日摂食嚥下リハ会誌　2002；6：65—72

Abstract

Videofluorographic evaluation of dysphagia in a patient with myasthenia gravis

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A 64-year-old woman with myasthenia gravis (MG) presented with isolated bulbar symptoms. Two years earlier, she had developed neck weakness, diplopia, and ptosis and was diagnosed with MG. Extensive thymectomy was performed, and she was treated with predonisolone (PSL). The neck weakness, diplopia, and ptosis improved over a 2-year period. However, dysphagia developed, and her voice took on a nasal tone that did not improve subjectively even after administration of 10 mg of edrophonium chloride (EC). We then performed videofluorography (VF). After consumption of 10 m*l* of liquid barium and 8 g of corned beef hash, she attempted to swallow, but the residue remained in the valleculae and the piriform fossa. After the EC injection, her dysphagia on ingestion of corned beef hash improved; however, there was slight subjective improvement in swallowing. Drinking of liquid barium resulted in some residue with slight improvement of dysphagia. After treatment with 70 mg of PSL for 4 weeks, VF showed improvement of dysphagia. Thus, VF, particularly during consumption of solid food, with EC administration is helpful in evaluating bulbar symptoms in patients with MG.

(Clin Neurol, 47: 669—671, 2007)

Key words: Myasthenia gravis, dysphagia, videofluorography, edrophonium test, chew and swallow

パーキンソン病の治療

薬物療法

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Clinical Neuroscience 別冊

Vol. 25 No. 1　2007年1月1日発行

中 外 医 学 社

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はじめに

今回は「Parkinson 病 What's new？」という特集であることから，パーキンソン病治療の一般論は成書にゆずり，ここでは，現在使用可能な抗パーキンソン病薬についての最近の話題，現在申請中の薬剤，および治験段階にある薬剤について述べたい．なお，治験段階の薬剤のうち，アデノシン A_{2A}受容体拮抗薬については別項に詳細に述べられているのでそちらを参照されたい．

パーキンソン病治療についての最近の話題

1．L-dopa 製剤の位置づけ

治療ガイドラインが相次いで発表された 2000 年前後は，パーキンソン病治療においてはドパミン受容体刺激薬の有用性が非常に強調され，L-dopa はむしろ悪者にされていた感がある．しかし，この 2 年ぐらいで国際的にも L-dopa の有用性が再評価されてきているように思われる．ターニングポイントは ELLDOPA study であろう．これは 2002 年マイアミでの Movement Disorder Society の年次総会の最終日に発表されたが，内容の重大性からか意外性からか論文としての発表は 2004 年になった[1]．

ELLDOPA study とは 1998〜2001 年に北米でなされた臨床研究で，パーキンソン病の診断後 2 年以内の初期例について，偽薬，L-dopa 合剤 150 mg，300 mg，600 mg/日の 4 群(各群約 90 名)の二重盲検にて，40 週間投与，その後薬物を中止し 2 週後まで UPDRS Ⅲで評価したものである．投与前に比較した改善度は投与中止 2 週間後でも L-dopa 投与群では偽薬群より有意によかった．半減期が 1 時間程度である L-dopa の直接効果が 2 週間も持続するはずもなく，L-dopa が神経保護作用をもつ，あるいは少なくとも L-dopa により神経回路網あるいは四肢の運動能力をより正常化することがよりよい状態を維持することにつながることが示唆され，少なくとも L-dopa は毒ではないことを示した．しかも，投与前後で行った β-CIT SCAN(ドパミントランスポーターを評価する SPECT)では L-dopa 投与群では量依存性に取り込みが低下していた．この結果は，それまでの PET，SPECT を用いたドパミン受容体刺激薬の神経保護作用を示唆するかもしれない研究[2,3](画像による神経終末の取り込み低下はドパミン受容体刺激薬のほうがよいが，臨床症状は L-dopa のほうがよい)の説得力をかなり弱める結果となった．

加えて，COMT 阻害薬である entacapone が北米，欧州で使用され，さらにドパ合剤＋entacapone の合剤が北米で認可されたこと，新たな MAOB 阻害薬である rasagiline の登場，rasagiline による「delayed start」[4]という新たな神経保護作用の評価方法による話題性などが加わり，最近は国際的にも「やはり L-dopa がパーキンソン病治療の golden standard である」というところに戻ってきている感がある．

2．各種ドパミン受容体刺激薬の差異

現在わが国で使用可能なドパミン受容体刺激薬は bromocriptine，pergolide，talipexole，cabergoline，pramipexole の 5 剤で，さらにごく最近 ropinirole が認可された．多数のドパミン受容体刺激薬のうちどれが一番よいかということになると，エビデンスレベルについてはその薬剤が開発された時代背景があるので多少の違いはあるが，集団として統計学的な有意差が出るほどの差はない[5,6]．たとえば，pramipexole のわが国での治験は偽薬および bromocriptine を対象として行われたが，実薬群はいずれも偽薬より優位に UPDRS Ⅲのスコアを改善したが，pramipexole と bromocriptine の間には有意差は認められなかった[7]．ただし，5 剤のドパミン受容体刺激薬は麦角剤，非麦角剤という構造の差異，半減期の差異などそれぞれ特徴があり，患者によりある薬剤では副作用が出現しやすいが他剤では出にくいなどの違いもあるため，選択肢が増えることはきわめて歓迎すべきことといえる．

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3．神経保護作用の評価について

2002年，2003年に相次いでpramipexole[2]，ropinirole[3]を用いたドパミン受容体刺激薬で治療を開始した群とL-dopaで治療を開始した群について，臨床症状とβ-CIT SPECTあるいはfluoro-DOPA PETによりドパミン神経終末を評価することにより，神経保護作用を評価するための大規模研究が発表された．これらはいずれも非常にきれいなデータでドパミン受容体刺激薬の神経保護作用が示されたかにみえたが，画像上明らかな差があるにもかかわらず，臨床効果には差がない，あるいはL-dopa開始群のほうがよいという結果で評価が難しくなった．さらに上記のELLDOPA study[1]が報告され，β-CIT SPECTやfluoro-DOPA PETでは薬物の神経保護作用を立証するのは困難という結論に至った．In vitroでは多くの薬剤の神経保護作用が報告され，臨床的に意味があるかどうかの評価は極めて重要である．その評価方法について，次に出てきたのがdelayed startという方法である．rasagiline 1 mg/日，2 mg/日，偽薬の3群で6ヵ月投与したのち，偽薬群は2 mg/日投与し，3群ともさらに6ヵ月間治療が継続され，UPDRS IIIで評価された．研究開始より12ヵ月後の時点で6ヵ月遅れてrasagilineが投与されたdelayed start群は，early start群にキャッチアップできなかったことから，rasagilineに神経保護作用がある可能性が示唆されると報告されている[4]．この方法が果たして本当に薬剤の神経保護作用を評価できるかはまだ議論のあるところで，今後も検討が必要である．

現在申請中の薬剤

1．ropinirole

わが国でも近日発売される予定の経口の非麦角系ドパミン受容体刺激薬である．pramipexoleにきわめて類似しているが，以下のようなちがいがある．すなわち，受容体親和性については，ドパミン受容体亜型への親和性はドパミン同様D 2>D 3で，pramipexoleほどD 3への親和性は突出していない．セロトニン系など他の受容体への親和性がきわめて低いのはpramipexoleと同様である．半減期は約6時間で，肝臓でグルクロン酸抱合などを受けた後，腎臓から排泄されるために，腎障害の影響は受けにくい[8]．臨床効果については多数のエビデンスがあり，L-dopa併用での効果は，insufficient dataでpossibly usefulではあるものの，単独使用，および運動合併症の予防，治療ともpramipexoleと同様，efficacious，clinically usefulと評価されている[5,6]．副作用の内容は，pramipexoleによく似ているが，これまでの偽薬対照試験での結果の解析では，ropiniroleはpramipexoleに比較して，幻覚の発現頻度は低いが，低血圧や眠気の出現頻度はやや高いという報告がある[9]．

2．entacapone

2007年にはわが国での承認が予想されるCOMT(catechol-o-methyltransferase)阻害剤である．もともとドパの主な代謝経路はドパ脱炭酸酵素(DDC)によりドパミンに変換されるものであるが，carbidopa，benserazideなどのDDC阻害剤との合剤が広く使われるようになり，COMTにより3-o-methyldopa(3 OMD)となる経路が活性化されるようになった．3 OMDは半減期が16時間と長いために，長期ドパ服用により血中濃度はむしろドパより3 OMDのほうが高くなる．3 OMDは腸管からの取り込みおよび血液脳関門の通過には，ドパと同じLNAA(Large Neutral Amino Acid)systemと呼ばれる能動輸送システムを使用するため，大量になりすぎるとドパの吸収を阻害する可能性もある．末梢でのドパの3 OMDへの代謝を阻害し，ドパの半減期を延長することを期待して開発された薬剤である．COMT阻害剤としてはtolcaponeが先行し高い効果をあげていたが，稀ではあるが重篤な肝障害が報告されたため，現在は北米で厳重な肝機能観察下のみで使用されている．entacaponeはすでに欧米で広く使用されているが重篤な肝障害は報告されていない．

entacaponeはその作用機序からドパとDDC阻害剤との合剤との併用で初めて効果を示す．単回投与ではドパの最高血中濃度(Cmax)は上昇せずに半減期が延長し，wearing-offの改善効果が期待できる[10]．なお，entacaponeは半減期がドパと同じく1時間程度と短いことから服用はドパと同時に服用するため，1日に3～4回多ければ8回の服用もありうる．単回投与でCmaxの上昇がないことから不随意運動は増加しないことが期待されるが，ドパの血中濃度があまり低くならないうちに次のドパを服用することから当然累積による血中濃度の上昇はおこり，off時間の減少とともに不随意運動もおこりうることになり，症状のコン

トロールにはこれまで以上にドパの服用量の調節が重要になるといえよう．それに伴い50 mgのドパ合剤が使用できることが望まれる．

これまでの海外の大規模治験では，ドパ服用と同時にentacapone 100〜200 mgずつ投与し(最高1日8回まで)off時間は1.6時間(偽薬は0.9時間)短縮した[11]．また，わが国では開発の予定は今のところないが新しいMAOB阻害薬のrasagilineと，entacapone，偽薬との並行群間比較試験(LARGO study)[12]では，rasagiline 1.0 mg/日とentacapone 200 mg/回(ドパと同時投与)にてほぼ同程度で偽薬に比較して有意な改善である，off時間の短縮(1.2時間)を認めた．いずれの試験においても不随意運動は多少増えても日常生活に問題になるほどではなく，ドパの減量で対応可能であるとされている．海外ではすでにドパ/carbidopa/entacaponeの合剤も発売されている．

3．zonisamide

zonisamideは抗てんかん薬としてわが国で開発された薬剤で，わが国では1980年代から，近年は欧米でも，難治性てんかんを対象に広く使用されている．われわれは偶然の臨床知見からzonisamideの抗パーキンソン作用を発見した[13]．すなわち，経過10年のパーキンソン病患者がたまたまてんかん発作をおこし，これに対する治療のためにzonisamide 300 mgを投与したところ，てんかん発作の消失とともにパーキンソニズムも著明に改善した．これをきっかけに小規模の臨床研究を行い，オープン試験でパーキンソン病の運動症状およびwearing-off現象の改善を認めた．また，この時点で1日50 mg程度の少量でも効果が明らかであること，半減期が長い薬剤(約63時間)であるので，1日1回投与で十分パーキンソニズムの改善を期待できることがわかった．その後，国内でL-dopa併用の進行期パーキンソン病患者(現在わが国で使用可能な抗パーキンソン病薬はすべて併用可能)を対象に製薬会社による大規模二重盲検試験を行い，50 mg/日(1日1回投与)でUPDRS IIIおよびwearing-offの改善を確認し[14]，現在申請中である．現在わが国で使用可能な抗パーキンソン病薬を使用して，なお治療効果が不十分なパーキンソン病患者を対象とした二重盲検試験では，罹患期間平均約9年で，UPDRS IIIで平均約6点の改善，1日のoff時間は1.5時間程度延長した．off時間の改善はCOMT阻害剤のentacaponeと同程度である．また，長期投与試験では，投与後4〜16週にかけて著明に改善し，その改善度は経過により，むしろより改善する傾向にあり，1年間持続した．平均罹患期間10年の患者で投与初期の改善のみならず，1年間にわたってより症状が改善する傾向にあることは重要な所見と思われる．

zonisamideは抗てんかん薬としては300〜600 mg/日が常用量であるが，抗パーキンソン効果はそれよりもかなり低い濃度で効果を呈する．抗てんかん薬としての作用機序は，Naチャンネル阻害とT型Caチャンネル阻害が報告されており，GABA系への直接作用はないとされている．抗パーキンソン効果の作用機序としては，基礎実験からzonisamideがドパミン合成の亢進，中等度のMAOB阻害作用を持つことが明らかになっている[15]．ただし，大規模二重盲検において，より強力なMAOB阻害剤であるseregilineとの併用の有無でzonisamideの効果が変化しなかったことから，MAOB阻害作用が本剤の効果の中心ではないと考えられる．

zonisamideは抗てんかん薬としての開発当初からradical scavengerとして神経保護作用がいわれていた．最近，浅沼らはパーキンソンモデルラットにドパを投与した際に惹起されるキノン体生成増加をzonisamideがほぼ完全に抑制することを報告しており，長期試験での効果改善作用と考え合わせて，神経保護という面でも期待できる[16]．

現在治験中の薬剤

1．rotigotine

非麦角系ドパミン受容体刺激薬の貼付薬で，現在後期第2相を施行中である．1日1回容量に応じて10 cm²(4.5 mg)〜30 cm²(13.5 mg)のパッチを貼付する．貼付剤であることから安定した血中濃度および効果が期待される．ただし，興味あることに貼付後4時間までは濃度上昇が鈍くTmaxは8時間である．また剝離後約3時間で50%の濃度まで低下することから，反復投与でも投与(貼付)後1〜2時間まで濃度が低下し，4〜8時間後まで徐々に濃度が上昇し，その後は24時間後まで濃度はほぼ一定である．受容体への親和性はD3が最も高く，pramipexoleと類似しており，pramipexole 1.5 mgとrotigotine 13.5 mgがほぼ同等とされている．代謝は硫酸抱合，グルクロン酸抱合が主