LINKAGE STUDY OF DOMINANTLY INHERITED OLIVO-PONTO-CEREBELLAR ATROPHY (OPCA) AND HOLMES' ATAXIA

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Summary   Both autosomal dominant olivo-ponto-cerebellar atrophy (OPCA) and Holmes' ataxia are the progressive neurodegenerative disorders of adulthood with unknown biochemical defects. In order to determine the genetic locus and possible genetic heterogeneity, linkage study was performed in 19 OPCA families comprising 180 individuals with 60 affected patients, and two Holmes' ataxia families comprising 39 individuals with 10 affected patients. By using computer program LIPED, linkage of each disorder was analyzed to 12 blood groups, 5 red cell enzymes, HLA-A, -B, -C, and F13A. No evidence suggesting linkage to these two disorders was obtained in the markers examined, including three 6p markers such as HLA, GLO1, and F13A. Furthermore, in 14 out of 15 HLA-informative OPCA families, negative lod scores for OPCA with HLA were obtained at most recombination fractions. Our results provide further evidence suggesting the genetic heterogeneity of dominant OPCA.
Key Words   spinocerebellar ataxia, HLA, linkage study, Holmes' ataxia, olivo-ponto-cerebellar atrophy

INTRODUCTION

Idiopathic hereditary spinocerebellar degeneration or ataxia (SCA) is a cluster

of neurodegenerative disorders.   Of these disorders, hereditary olivo-ponto-cerebellar atrophy (OPCA Menzel type) and Holmes' hereditary cerebello-olivary degeneration (Holmes' ataxia) are the main disorders showing autosomal dominant inheritance with a relatively late age of onset (usually fourth to fifth decades).   However, the clinical features and course, and the distribution of pathological alteration are quite different from each other, and these two disorders are considered to be genetically different entities.   Main clinical manifestations of dominant OPCA are cerebellar ataxia associated with limb spasticity, amyotrophy, and sings and symptoms of cranial nerves and basal ganglia involvements.   Pathologically, neuronal degeneration and gliosis are observed in cerebellar cortex, deep cerebellar nuclei, brain stem, spinal cord, and basal ganglia.   In contrast, Holmes' ataxia shows only cerebellar ataxia, and pathological alterations are confined to cerebellar cortex and olivary nucleus (Harding, 1984).   Therefore, clinical differentiation between OPCA and Holmes's ataxia is possible with considerable certainty during life.

In both OPCA and Holmes' ataxia, little is known about biochemical defects. The causing genes for OPCA and Holmes' ataxia are not known but the first possible linkage of OPCA was reported with HLA markers on the short arm of chromosome 6 (Yakura et al., 1974).   After their report, Jackson et al. (1977) reported the evidence that OPCA locus is located at 12 cM apart from HLA locus.   In subsequent studies, the linkage has been investigated between OPCA and genetic markers on 6p (Jackson et al., 1978; Möller et al., 1978; Wastiaux et al., 1978; Pedersen et al., 1980; Nino et al., 1980; Whittington et al., 1980; Morton et al., 1980; Koeppen et al., 1981; Haines et al., 1984; Haines and Trofatter 1986; Kumar et al., 1986; Rich et al., 1987; Wilkie et al., 1987; Kanazawa et al., 1987; Zoghbi et al., 1987, 1988).   These data show that the locus for OPCA is on 6p24-p21.3 (The first OPCA locus mapped to 6p has been now defined as SCA1.   See HGM9; Olaisen et al., 1987).   However, linkage analysis on additional families is necessary to determine if there is more than one OPCA locus, and whether the Holmes' ataxia is linked to HLA.   Since the existence of genetic heterogeneity would be important for future diagnosis, isolation and characterization of the OPCA and Holmes' ataxia genes, we have begun genotyping a large collection of OPCA and Holmes' ataxia families.   In this paper, we report the preliminary results of genotyping 19 OPCA and 2 Holmes' ataxia families with HLA and other genetic loci.   Our data provide further evidence suggesting the existence of genetic heterogeneity of dominant OPCA.   In addition, this is the first report for a linkage study of Holmes' ataxia.

MATERIALS AND METHODS

Family resources.   The 21 OPCA and Holmes' ataxia affected families used in this study were contributed by the Department of Neurology of Hokkaido University School of Medicine, Hokuyukai Neurological Hospital, Sapporo Minami National Sanatory Hospital, and Kushiro Rosai Hospital.   Data of the date of birth, age

of onset of the disease, and the number of children were collected in those families as much as possible. All of the patients were examined by one of the authors (H.S.). The determination of clinical diagnosis is based on the positive family history, typical clinical manifestations of the affected members, and pathognomonic alterations of brainstem and cerebellar atrophies observed on X-ray computed tomography or magnetic resonance imaging. The clinical diagnosis has been confirmed pathologically in 2 families, P.2 and P.16. Clinico-pathological features of autopsied cases in these 2 families have been reported by Fujii *et al.* (1987) and Kudoh *et al.* (1986), respectively. In the other 17 OPCA families and 2 families of Holmes' ataxia, the clinical diagnosis has not been confirmed pathologically yet.

Taking the possible heterogeneity of OPCA into account, differential diagnosis of OPCA from other related disorders were carefully performed. In our OPCA patients, no cases manifesting dystonia were observed, so far examined. Therefore, Joseph disease, a clinical subtype of dominant OPCA (Rosenberg, 1986), was not included in the present OPCA families. Dentato-rubro-pallido-Luysian atrophy (DRPLA), particularly ataxochoreoathetoid form, was the another disorder which must be differentiated from dominant OPCA. In this regard, it has been known that choreic or athetotic involuntary movement is occasionally observed in hereditary OPCA (Harding, 1984). In the present OPCA families, 2 cases in family P.2 showed choreic movement. However, cerebellar ataxia and pyramidal tract involvement predominate the clinical pictures of the other patients of this family, which is consistent with the diagnosis of hereditary OPCA. Overall, the clinical features of the present patients were within the clinical spectrum of dominant OPCA.

The pedigrees were compiled on the basis of informations provided by extensive search for family members. It was left "uncertain" when the mode of inheritance could not be established due to the early death of parent. The affected families have now been continuously collected. In this report, the families available of linkage informations were analyzed. These 19 OPCA families comprised 180 individuals, with 60 affected subjects. The two Holmes' ataxia families comprised 39 individuals with 10 affected patients (Table 1 and Fig. 1). The pedigree data of these 21 SCA families (P.1 to P.21) were now available, spanning 6 generations in the largest pedigree (P.1), and 3 generations in the small ones.

Determination of genetic markers. Blood samples were collected from each individual for determination of genetic markers and lymphoblastoid cell transformation. The genetic markers examined in this study and their current chromosomal assignments were presented in Table 2.

Blood-group typing. After blood sampling, the 12 blood groups were serotyped using standard methods. They include ABO, Rh(CcDdEe), Lewis, MNSs, Diego, Kell, Duffy, Kidd, Lutheran, Xg, Jr, and P.

HLA typing. The standard NIH microlymphocytotoxicity test was used for HLA-A, -B, -C typing.

Enzymes. Hemolysates, stored in a deep freezer, were used as a sample for

Table 1. Families affected with hereditary spinocerebellar ataxia.

| Pedigrees	Disease[a]	Total number (M/F)[d]	Patients (M/F)[d]
P. 1	OPCA	16/23	9 (5/4)
P. 2	OPCA	13/10	6 (4/2)
P. 3	OPCA	1/ 6	3 (0/3)
P. 4	OPCA	3/ 5	4 (0/4)
P. 5	OPCA	2/ 1	2 (1/1)
P. 6	OPCA	1/ 6	3 (1/2)
P. 7	OPCA	1/ 4	3 (0/3)
P. 8	OPCA	3/ 4	4 (2/2)
P. 9	OPCA	2/ 3	2 (0/2)
P. 10	OPCA	1/ 4	2 (0/2)
P. 11	OPCA	3/ 2	1 (1/0)
P. 12	OPCA	2/ 5	2 (1/1)
P. 13	OPCA	1/ 3	2 (0/2)
P. 14	OPCA	6/ 3	2 (2/0)
P. 15	OPCA	4/ 5	3 (2/1)
P. 16	OPCA	4/ 2	2 (2/0)
P. 17	OPCA	9/ 5	4 (4/0)
P. 18	OPCA	5/ 7	4 (1/3)
P. 19	OPCA	4/ 1	2 (2/0)
P. 20	Holmes	4/ 7	2 (0/2)
P. 21	Holmes	12/16	8 (7/1)
Total	OPCA 19 families	180 (81/99)	60 (28/32)
	Holmes 2 families	39 (16/23)	10 (7/3)

[a] OPCA, olivo-ponto-cerebellar atrophy (Menzel type); Holmes' ataxia, Holmes' hereditary cerebello-olivary atrophy.   [b] M, male; F, female.
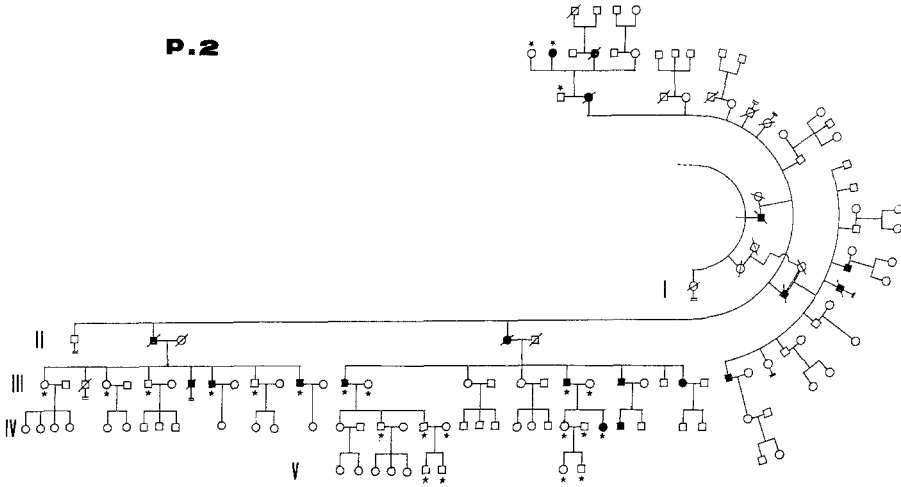
Table 2. Genetic markers used for linkage study.

Marker	Chromosomal assignment[a]
ABO	9q34.1-q34.2
Rh	1p36.2-p34
MNS	4q28-q31
Diego	unknown
Kidd(Jk)	18q11-q12
Duffy(Fy)	1p21-q23
P	6
AcP1	2p25 or p23
EsD	13q14
6PGD	1p36.2-p36.13
GLO1	6p21.3-p21.1
PGM1	1p22.1
HLA	6p21.3
F13A	6p24-p21.3
(SCA1)	(6p24-p21.3)

[a] From HGM9.
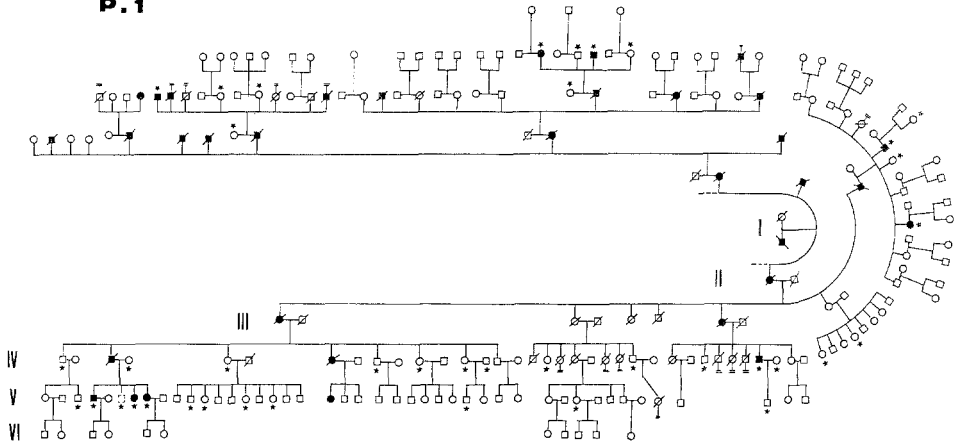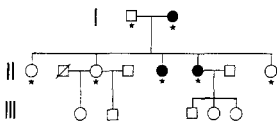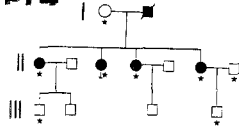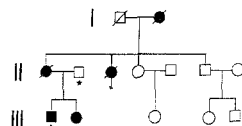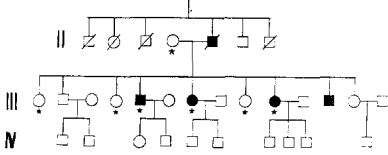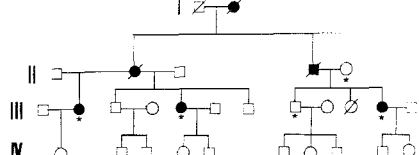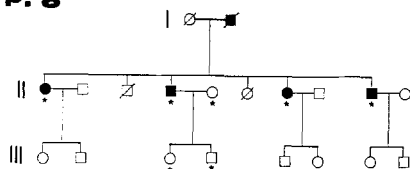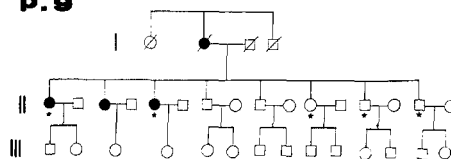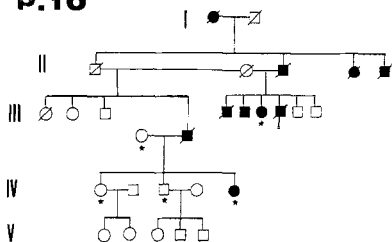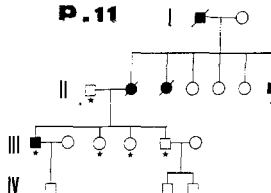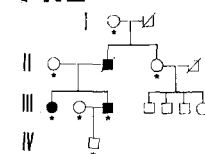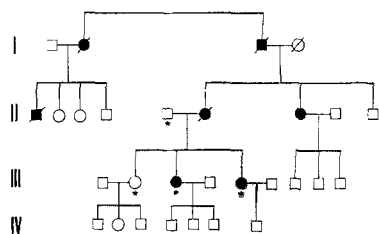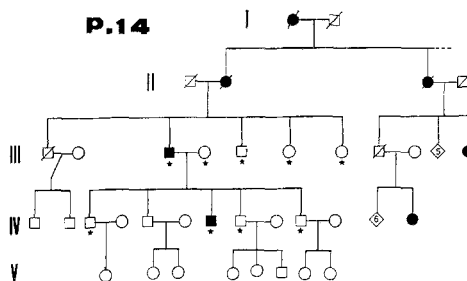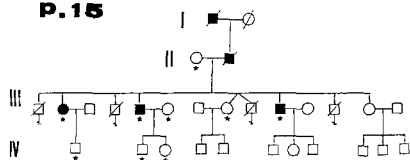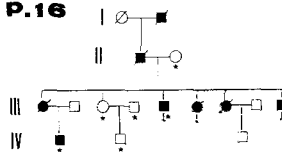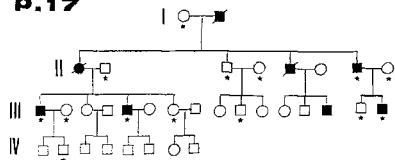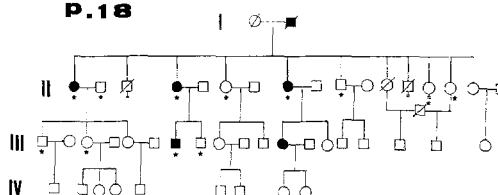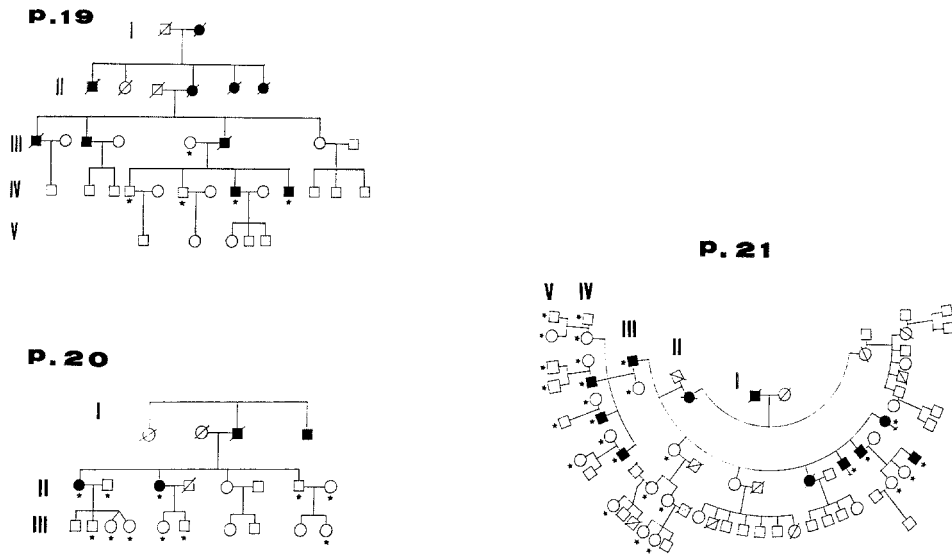
Fig. 1. The family pedigrees of dominant spinocerebellar ataxia. The deduced pedigrees of 21 different families with dominant ataxias are presented. P. 1 to P. 19 are the families with dominant OPCA. P. 20 and P. 21 with Holmes' ataxia. In the pedigrees, generations are expressed as Roman numerals. Deceased individuals are shown by slash. Males are indicated by squares, females by circles, and affected patients are shown by filled in symbols. Individuals examined in this investigation are indicated with filled stars.

enzyme electrophoresis. Isoenzymes of glyoxalase-1 (GLO1), esterase D (EsD), 6-phosphogluconate dehydrogenase (6PGD), red cell acid phosphatase (AcP1), were analyzed by starch gel electrophoresis, as described by Harris and Hopkinson (1976). Phosphoglucomutase-1 (PGM1) was examined by the isoelectric instrument focusing method of the manual of LKB (Sweden).

Coagulating factor XIIIA (F13A). According to the monodansyl cadaverine-casein functional detection method of Board (1979), polymorphism of F13A was determined after high-voltage agarose gel electrophoresis of plasma samples.

Linkage analysis. Linkage analysis was performed using the computer program LIPED with the correction of age-dependent penetrance (Ott, 1974); the lod score over +3 is regarded as a proof of close linkage, while the value below −2 is strong evidence of non-linkage at given recombination fractions. In case of the HLA antigen, haplotypes were scored as a single unit, according to the instruction of Ott (1978). Because of the dominant trait of these disorders, it was assumed that all the patients were heterozygotes. The age of onset was defined as the age when the patient first noticed clinical symptoms. Mean±SD of the age at onset were

inputted at computation of the lod scores of hereditary OPCA and Holmes' ataxia, respectively.   The penetrance was assumed to be 100%.

RESULTS

Pedigree data

Nineteen families with hereditary OPCA and 2 families with Holmes' ataxia were selected for genetic linkage analyses (Table 1 and Fig. 1).   The 19 OPCA families (P.1 to P.19) comprised 180 individuals, including 60 affected patients who are still alive.   The 2 families with Holmes' ataxia (P.20 and P.21) comprised 39 individuals, with 10 affected subjects.   Interrelationship within these family pedigrees is anknown at present.

Major clinical features of the affected members in each OPCA family were fairly uniform with cerebellar ataxia, nystagmus, hyperactive deep tendon reflexes, and positive Babinski and/or Chaddock signs.   Facial and/or lingual muscle twitching, neck flexor weakness, optic disc pallor, mydriasis, upward gaze palsy, dysphagia, limb spasticity, limb muscle atrophy, or impaired vibratory perception were also observed in parts of advanced patients.   In four families, P.2, P.11, P.12, and P.16, characteristic disturbance of pursuit eye movement, known as "slow eye movement" or slow saccade, was observed in parts of affected patients.   Two patients in P.2 showed choreic movement, and one of them showed marked neurogenic limb muscle atrophy.   Rhythmic myoclonus was observed in a case of family P.2 and P.12, respectively.

As shown in Table 1 and Fig. 1, affected subjects were distributed throughout more than two generations in both OPCA and Holmes' ataxia.   There were 28 males and 32 females affected by OPCA (male : female ratio=0.88), and 7 male and 3 female by Holmes' ataxia (ratio=2.3).   In every generation, each family had affected individuals except for those younger than the age of onset or dead with non-neurological diseases or by accident.   Of informed patients, mean age of onset was 32.7 ± 10.3 (n=57, ranged from 17 to 56 years old) in OPCA, and 36.0 ± 5.4 (n=9, ranged from 26 to 46 years old) in Holmes' ataxia.   This fact implies that most of carriers manifest symptoms after the critical age at onset.   This mode of inheritance, no sexual preference, and the age of onset clearly indicate that these two ataxic disorders are inherited in an autosomal dominant trait with age-dependent penetrance.

Linkage analysis of OPCA

Genotyping 19 OPCA-affected families, comprising 180 individuals, resulted in 15 families informative for HLA markers.   The results of the OPCA-HLA linkage analysis are presented in Table 3.   The maximum lod score was 0.868 (θ=0.000) in P.16.   In the other families, even in two large families (P.1 and P.2), negative lod scores were found at most recombination fractions.   These results demonstrated no

Table 3. Lod score for OPCA and HLA loci.

Pedigree	Recombination fraction (θmf)[a]					
	0. 000	0. 050	0. 100	0. 200	0. 300	0. 400
P. 1 (68)[b]	−14. 400	−2. 117	−1. 114	−0. 224	0. 098	0. 141
P. 2 (30)	−19. 348	−4. 379	−2. 994	−1. 584	−0. 806	−0. 315
P. 3 (7)	−4. 562	−0. 844	−0. 538	−0. 244	−0. 097	−0. 023
P. 4 (10)	−3. 026	−0. 411	−0. 182	−0. 025	0. 013	0. 011
P. 5 (4)	−3. 683	−0. 721	−0. 444	−0. 194	−0. 076	−0. 018
P. 7 (4)	−0. 648	−0. 429	−0. 298	−0. 142	−0. 058	−0. 014
P. 8 (9)	−3. 100	−1. 438	−0. 889	−0. 390	−0. 153	−0. 037
P. 10 (9)	−0. 108	−0. 085	−0. 066	−0. 036	−0. 016	−0. 004
P. 11 (6)	−1. 317	−0. 575	−0. 350	−0. 147	−0. 055	−0. 127
P. 12 (9)	−8. 032	−1. 746	−1. 186	−0. 664	−0. 380	−0. 177
P. 14 (11)	−2. 799	−0. 891	−0. 419	−0. 021	0. 112	0. 108
P. 15 (13)	−0. 273	−0. 148	−0. 078	−0. 018	−0. 006	−0. 006
P. 16 (9)	0. 868	0. 782	0. 691	0. 499	0. 302	0. 124
P. 17 (16)	−11. 918	−1. 125	−0. 589	−0. 137	0. 035	0. 069
P. 18 (15)	−9. 332	−3. 014	−2. 064	−1. 071	−0. 519	−0. 185
Total	−81. 677	−17. 141	−10. 521	−4. 399	−1. 605	−0. 337

[a] θmf, sex-combined lod score. [b] Number within parenthesis indicates the total members of the family; it includes both the members alive and the deceased ones whose genotypes are deduced from family pedigree.

Table 4. Sex-specified lod scores for F13A:HLA, F13A:OPCA, and HLA:OPCA linkages.

Locus pair	Recombination fraction (θ)				
	0. 050	0. 100	0. 200	0. 300	0. 400
Paternal lod score					
F13A:HLA (14)[a]	−1. 435	−0. 072	0. 697	0. 615	0. 258
F13A:OPCA (11)	−1. 667	−1. 033	−0. 356	−0. 034	0. 068
HLA:OPCA (12)	−11. 840	−7. 530	−3. 346	−1. 299	−0. 294
Maternal lod score					
F13A:HLA (13)	−4. 400	−2. 592	−1. 014	−0. 377	−0. 111
F13A:OPCA (8)	−0. 543	−0. 173	0. 150	0. 163	0. 060
HLA:OPCA (10)	−5. 301	−2. 991	−1. 053	−0. 307	−0. 043

[a] Number of informative pedigrees.

evidence of close linkage between the gene causing OPCA and HLA gene.   When the lod scores of all pedigrees were pooled, OPCA-HLA linkage was excluded at the recombination fraction from 0.000 to 2.500.

Since the SCA1 locus is assigned telomeric to HLA locus and in order to validate the procedure, paternal lod score (θm) and maternal lod score (θf) of F13A:OPCA, HLA:OPCA, and F13A:HLA linkage were calculated, respectively. As shown in Table 4, paternal F13A:HLA lod scores were positive, but not so high to support linkage; maternal F13A:HLA lod scores were negative at all recombination fractions. Slightly positive values were obtained in F13A:OPCA lod score, but the peak values of both sexes were not significant. Both maternal and paternal lod scores for HLA: OPCA linkage were negative at all recombination fractions. In paternal lod scores, the lod scores for HLA:OPCA linkage were far negative than that for F13A:HLA linkage throughout all recombination fractions.

In other genetic loci shown in Table 5, the lod scores for ABO, Diego, Jk, Fy, AcP1, 6PGD, and GLO1 were negative throughout all recombination fractions, whereas the lod scores for linkage between OPCA and Rh, MNS, P, EsD, PGM1, and F13A were slightly positive at higher recombination fractions, but the values were too small to suggest linkage between OPCA and these loci. These genotyping data showed no conclusive evident for linkage of OPCA to these 14 genetic loci, even to GLO1 and F13A on 6p.

Linkage analysis of Holmes' ataxia
As shown in Table 6, the two families of Holmes' ataxia (P.20 and P.21), showed negative lod scores for all recombination fractions in ABO, MNS, Diego, GLO1, 6PGD, EsD, and HLA. Slightly positive lod scores were found *versus* P, PGM1,

Table 5.   Lod scores between OPCA and 13 markers.

Marker	Recombination fraction (θmf)[a]					
	0. 000	0. 050	0. 100	0. 200	0. 300	0. 400
ABO	−18. 504	−3. 485	−2. 141	−0. 869	−0. 298	−0. 065
Rh	−14. 651	−1. 589	−0. 602	0. 016	0. 097	0. 051
MNS	−28. 351	−3. 644	−2. 141	−0. 765	−0. 159	0. 054
Diego	−15. 158	−3. 731	−2. 113	−0. 639	−0. 130	−0. 024
Jk	−18. 215	−2. 866	−1. 715	−0. 697	−0. 232	−0. 028
Fy	−6. 631	−0. 719	−0. 413	−0. 123	−0. 014	−0. 005
P	−14. 357	−1. 000	−0. 283	0. 203	0. 265	0. 150
AcP1	−44. 560	−7. 591	−4. 851	−2. 195	−0. 894	−0. 251
EsD	−11. 840	−1. 130	−0. 376	0. 085	0. 102	0. 010
6PGD	−4. 470	−0. 717	−0. 479	−0. 241	−0. 119	−0. 055
GLO1	−26. 517	−3. 466	−1. 717	−0. 450	−0. 109	−0. 060
PGM1	−29. 135	−3. 671	−1. 641	−0. 125	0. 200	0. 110
F13A	−9. 988	−2. 288	−1. 204	−0. 182	0. 142	0. 132

[a] θmf, sex-combined lod score.

Table 6. Lod scores between Holmes' ataxia and 12 markers.

Marker	Recombination fraction (θmf) [a]					
	0.000	0.050	0.100	0.200	0.300	0.400
HLA	−15.962	−4.763	−3.083	−1.510	−0.706	−0.245
ABO	−7.858	−1.761	−1.004	−0.383	−0.133	−0.245
MNS	−0.691	−0.496	−0.355	−0.172	−0.069	−0.016
Jk	0.622	0.552	0.478	0.325	0.173	0.050
P	−0.039	−0.025	−0.014	−0.003	0.000	0.000
Diego	−0.549	−0.283	−0.161	−0.053	−0.015	−0.003
GLO1	−0.238	−0.185	−0.141	−0.075	−0.032	−0.008
PGM1	−4.953	−0.327	−0.111	0.016	0.027	0.010
6PGD	−5.365	−1.075	−0.602	−0.180	−0.039	−0.004
EsD	−7.919	−2.228	−1.387	−0.613	−0.241	−0.057
AcP1	0.121	0.102	0.084	0.050	0.024	0.006
F13A	0.001	0.001	0.003	0.002	0.001	0.000

[a] θmf, sex-combined lod score.

AcP1, Jk, and F13A. These data showed no evidence of linkage between Holmes' ataxia and HLA and other loci here examined.

Clinical difference between HLA-linked and HLA-nonlinked OPCA

If any of HLA-linked OPCA were included in the present 15 HLA-informative OPCA families, only P.16 was likely to be linked to HLA. However, the pedigree size and the number of individuals of P.16 was so small that it was impossible to discriminate true HLA-OPCA linkage from HLA haplotype segregation by chance. In this family, 2 patients examined by one of the authors (H.S.) and other cases reported by Kudoh *et al.* (1986) clinically manifested slow eye movement, limb muscle atrophy, positive Babinski and/or Chaddock sign with minor leg spasticity, and terminal mental decline. However, these clinical features were also observed in the part of patients of HLA-nonlinked families, such as P.2, P.11, and P.12. The predominant clinical picture of other HLA-nonlinked families was rather uniform, showing dysarthria, nystagmus, dysphagia, ataxia, and leg spasticity with positive Babinski and/or Chaddock sign. From these available information, we have not found any clinical difference between the two type of OPCA families.

DISCUSSION

Determination of each gene causing OPCA and Holmes' ataxia to a specific chromosome or chromosomal region should permit the subsequent isolation of the

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gene and the identification of the aberrant protein, with implications for the treat-
ment, the prevention, and early clinical detection of these disorders.   In such a
study, it is essential to find closely linked genetic markers to the genetic defect and
the heterogeneity within the disorder.   The pilot linkage study of dominant OPCA
was performed by Schut (1951), however, because of a few polymorphic markers
used, no markers linked to OPCA gene were found.   Since the first report of a pos-
sible linkage between dominant OPCA and HLA genes on chromosome 6 (Yakura
et al., 1974), a number of workers have studied the linkage of OPCA to HLA and
other genetic markers on the short arm of chromosome 6 (6p) (Jackson et al., 1977,
1978; Möller et al., 1978; Wastiaux et al., 1978; Pedersen et al., 1980; Nino et al.,
1980; Whittington et al., 1980; Morton et al., 1980; Koeppen et al., 1981; Haines
et al., 1984; Kumar et al., 1986).   The accumulation of evidence has dis-
closed that there are HLA-linked and HLA-nonlinked families in OPCA.   The
OPCA locus in the "HLA-linked families" have now termed SCA1 and assigned to
6p24-p21.3 (Haines and Trofatter, 1986; Wilkie et al., 1987; Kanazawa et al., 1987;
Zoghbi et al., 1987, 1988).   Recent analyses by Zoghbi et al. (1987, 1988) and Wilkie
et al. (1987) have demonstrated that recombination frequency between SCA1 and
HLA is 0.12 and 0.17, respectively.   The SCA1 is mapped centromeric to the gene
for F13A (Wilkie et al., 1987) or telomeric to HLA (Haines and Trofatter, 1986;
Kanazawa et al., 1987).   Based on the multipoint linkage analysis, Rich et al. (1987)
in the same group of Wilkie et al. (1987) have reported that the gene order is HLA-
A:SCA1:D6S7:F13A:6pter.   However, in the present study of 15 HLA-informative
OPCA families, we obtained negative evidence for linkage of OPCA to HLA, GLO1,
and F13A on 6p, and to any of the genes tested.   Although additional OPCA loci
cannot be determined, these results indicate probable genetic heterogeneity of OPCA,
which is linked and nonlinked to HLA.

   In HLA-linked and HLA-nonlinked OPCA families, Pedersen et al. (1980) and
Kumar et al. (1986) reported that there are certain differences in the clinical features,
such as severity of pyramidal tract involvement, mental decline, or pigmentary retinal
degeneration.   In his recent classification of dominant OPCA, Harding (1984)
discriminated OPCA with pigmentary retinal degeneration from OPCA without it.
Correlation of SCA1 to this OPCA classification needs further accumulation of
evidences.   However, none of the cases in the present study showed pigmentary
retinal degeneration.   In our OPCA families, only 1 (P.16) out of 15 HLA-informative
families were segregated with HLA haplotype.   However, pedigree size and the
number of subjects of P.16 were too small to ascertain true linkage.   Clinically, it
was difficult to discriminate this pedigree (P.16) from HLA-nonlinked pedigrees,
because clinical manifestations quite similar to P.16 were observed in parts of other
HLA-nonlinked pedigrees.   In literature, Schut-Swier kindred is the large HLA-
linked pedigree which has been genetically studied by Schut (1951), Haines et al.
(1984), Haines and Trofatter (1986), Rich et al. (1987), and Wilkie et al. (1987).   The
clinico-pathological findings of this kindred have also been reported by Schut (1950,

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1951) and Landis *et al.* (1974). A unique sign of "slow apractic eye movement," as described in this kindred (Landis *et al.*, 1974), is also observed in our P.16 family but also in parts of our HLA-nonlinked families. When compared HLA-nonlinked OPCA pedigrees of ours and those of Wastiaux *et al.*, (1978) to Shut-Swier kindred as well as to the HLA-linked one of Nino *et al.* (1980), it seems impossible to find any apparent clinical differences between the two types of hereditary OPCA. It is partly because of considerable phenotypic heterogeneity even in single but large pedigrees, as observed in Schut-Swier kindred (Schut, 1950; Landis *et al.*, 1974). At present, it is still uncertain whether there are significant clinical differences, as suggested by several investigators (Pedersen *et al.*, 1980; Kumar *et al.*, 1986), between HLA-linked and -nonlinked OPCA pedigrees. These facts indicate that the phenotypic variation itself cannot be reliable indicator for genetic heterogeneity at OPCA locus.

Autosomal dominant OPCA is currently regarded to be a established clinicopathological entity. However, chiefly because of variation in phenotypes, there have been a matter of debates in the classification of clinical subtypes. Generally speaking, affected individuals in a single family usually tend to have similar clinical features and courses. However, generation anticipation of earlier onset and higher severity of the disease is noted in the descendant generations. Even in a single family, marked variations of clinical features are sometime observed. In addition to this, the extent of pathological involvement also vary from patient to patient in a single family, as well as among separate families. Therefore, a comprehensive picture of the disease could not emerge from the study of insufficient size of affected families, such as most of ours and reported ones. This fact should be taken into consideration when evaluating the correlation of clinical features to previous OPCA-HLA linkage results. Furthermore, it indicates that even a single locus of OPCA can produce considerable phenotypic variations. At present, it is not known whether phenotypic heterogeneity within or between affected families is due to multiple loci, multiple alleles, or interaction of modifier genes.

For another prediction for the conflicting results of linkage study of OPCA, pedigree structure, in particular, size and distribution of affected members are considered. However, it is unlikely that nonlinkage to HLA is due to the pedigree structure of affected families, because our families and those of reported ones include sufficient large pedigrees to prove HLA linkage. Even small family pedigrees, theoretically summation of lod scores can prove linkage. The second possibility is that those conflicting results reflect the different recombination rate of investigated families. However, no supportive evidence has been reported. The third possibility in the failure of demonstration of OPCA:HLA linkage is the altered recombination rate in geographically isolated and genetically homogeneous populations, particularly such as Japanese. Recently demonstrated linkage of F13A:HLA (Wong *et al.*, 1988) has not been reconfirmed not only in our study but also in the extensive study of Nishigaki *et al.* (1986), even by using sufficient number of pedigrees. The discrep-

ancy of this linkage data between these of Japanese and those in English literature implies that, for instance, OPCA:HLA nonlinkage obtained in our study reflects the potentially higher recombination rate occurring in Japanese than expected. Although we took this possibility into account, far negative values observed in the paternal lod scores for HLA:OPCA linkage than those for F13A:HLA linkage seem against this possibility (Table 4). When taken our results and previous genetic linkage studies into consideration, it is most likely at the present time that there is genetic heterogeneity in dominant OPCA, as suggested by several authors both genetically and clinically (Wastiaux *et al.*, 1978; Pedersen *et al.*, 1980; Whittington *et al.*, 1980; Morton *et al.*, 1980; Koeppen *et al.*, 1981; Kumar *et al.*, 1986). If there is genetic heterogeneity in OPCA, it implies that simple summing of lod scores in OPCA linkage studies of different families will make the linkage obscure. For this reason, it is reliable, if possible, to obtain linkage information from a single large family rather than from many different small families, as already mentioned (Ott, 1985). Until now, no markers other than HLA have been documented to be linked to SCA1 locus by two-point linkage analysis. In order to solve the discrepancy in OPCA:HLA linkage studies and also to confirm the possible genetic heterogeneity of OPCA, it is necessary to find a set of polymorphic 6p markers other than HLA which must be more tightly linked to SCA1 locus than HLA. In addition, ruling out of the localization of HLA-nonlinked OPCA locus on chromosome 6p by systematic linkage study is essential to prove genetic heterogeneity of OPCA.

In contrast to OPCA, Holmes' ataxia is a distinct clinico-pathological entity in hereditary SCA. However, systematic genetic linkage studies of Holmes' ataxia have not been performed. Koeppen *et al.* (1981) investigated GLO1, Bf, and HLA in an affected family (pedigree 1 in their report), but found no evidence of linkage. Our results have not indicated any conclusive evidence of linkage between Holmes' ataxia and the test markers. In order to estimate the location of the locus of Holmes' ataxia, it is necessary to collect further informative families as well as to perform continuous genetic linkage studies.

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