厚生労働科学研究費補助金
難治性疾患克服研究事業

生体試料の収集に関する研究：
日本人多発性硬化症患者緯度・病型別臨床データ／サンプルバンクの構築

平成21年度　総括研究報告書

研究代表者　吉良　潤一

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課題番号：　H21－難治－一般－078

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1. 研究目的

日本人多発性硬化症（multiple sclerosis; MS）患者を対象に，臨床・MRI データ所見，ヒト白血球抗原（human leukocyte antigen; HLA）クラス II アリル，抗アクアポリン 4（aquaporin 4; AQP4）抗体の有無から病型分類し，病型ごとの血清・髄液・細胞・DNA バンクを，緯度により南日本，中央日本，北日本コホートとして構築することを目的とする。各コホート間の共同研究のみならず，国内外の MS 研究者への情報公開や試料提供，他の MS コホートとの共同研究が可能な体制を構築する。

2. 研究方法

評価スケール標準化の後に，南西日本の協力施設から MS 患者をバンクに登録し，臨床データ・試料の収集を行い南日本 MS コホートとする。併せて患者生育環境調査を実施し，十分なサンプル数が集積された段階で高感度抗 AQP4 抗体測定を行う。臨床・抗体検査・MRI データに基づいた MS 病型分類を行い，病型ごとに HLA クラス II アリル間の epistatic interaction を解析する。また他地域でも同様な MS コホートを立ち上げるための方策，各コホート間の共同研究の実施のあり方などを検討し，全国的な MS バンクの構築を目指す。

（倫理面への配慮）

必要とされる倫理的課題については，班員所属施設の倫理委員会・遺伝子解析倫理委員会の承認を得る。特に研究対象者に対する不利益，危険性やインフォームドコンセントに配慮する。

3. 研究結果及び報告

当バンクにて収集した臨床データ，試料については全国的に円滑かつ倫理上の配慮をもって利用できるよう「難治性疾患克服のための難病研究資源バンク開発研究」事業である難病研究資源バンク（基盤研）に提供することとした。難病研究資源バンク（基盤研）と協力して，各施設で収集された検体の難病研究資源バンク（基盤研）への提供を可能とするための申請書を作成した。現在，研究代表者，分担者が所属している倫理委員会にて審査が行われている。認可後は試料を継続して難病研究資源バンクに移送する。今年度は研究期間が限られていたため，難病研究資源バンク（基盤研）への試料提供体制の構築にとどまったが，今後は継続的に試料の収集と提供が可能となる見通しである。

4. 評価

1) 達成度について

西日本の 6 大学神経内科（九州大，大阪大，近畿大，広島大，山口大，愛媛大）で匿名化した MS 患者 287 例，健常対照者 303 例の DNA を収集した。患者の HLA クラス II アリルの genotyping，高感度 AQP4 抗体測定法により，大部分の例では，抗 AQP4 抗体の有無を決定した。各施設で臨床，画像所見についての情報収集が進められ，データベースを構築している。

収集した検体利用の道筋については，難病研究資源バンクを利用することとなり，準備段階を終了したと考える。

2) 研究成果の学術的・国際的・社会的意義について

本研究により日本人では初めての大規模な MS データベースが構築され，前向きに標準化された臨床データが収集され病型ごとの自然経過，IFNβ 投与下の治療経過を解明するための基礎的土台が構築されたと考える。また疾患頻度が低いために困難であった MS に関する遺伝子解析が，全国的に収集され多施設で利用可能となることで，遺伝的特徴についての解析が発展することが期待される。人種による遺伝的多型と疾患に影響する多型の関連が世界的にも問題となっており，日本人を対象とした遺伝子

バンクの構築とその解析は，国際的にも意義は高い。また根治療法のない MS 患者は，いったん発病すると生涯にわたって再発に苦しみ重い機能障害を残す。本研究により，日本人における MS 発症リスク因子，防御因子が解明されれば，増加し続ける MS の発症予防につながる施策の実施が期待できる。

3) 今後の展望について

画像所見などについては所属施設間で意見の統一を図り，登録データについてのガイドラインを策定する。バンク登録後の検体についてはその存在を周知し，広範な研究利用を促すことで，大きな成果が期待できる。遺伝子解析については HLA に焦点をあて，発症リスク，病型，画像所見，治療との関連を検討する。

4) 研究内容の効率性について

当研究では統一的なデータベースの構築と検体利用の基盤をつくることで，多くの研究者に MS の臨床像と遺伝的関連を解析する機会を提供することが可能となり，日本人 MS の研究効率を多いに高めると考えられる。またこれまでの研究は効率的に予算内で行うことができた。

5. 結論

MS 患者データを収集し匿名化された臨床，画像データ，試料の収集を行った。収集検体の HLA クラス II アリルの解析，抗 AQP4 抗体の測定を行った。

各施設で難病研究資源バンクへの試料移送のための倫理審査を行い，今後のバンク構築に向けてのコンセンサスを得た。

6. 研究発表

1) 国内

口頭発表	40 件
それ以外（レビュー等の発表）	23 件

そのうちの主なもの

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そのうちの主なもの

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なし

研究成果の刊行に関する一覧表

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RESEARCH PAPER Multiple Sclerosis 2009; 15: 159–173

Temporal changes and geographical differences in multiple sclerosis phenotypes in Japanese: nationwide survey results over 30 years

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Background There are two distinct phenotypes of multiple sclerosis (MS) in Asians, manifesting as optic-spinal (OSMS) and conventional (CMS) forms. In Japan, four nationwide surveys of MS have been conducted. The first three were in 1972, 1982, and 1989, and we performed the fourth in 2004.
Results The recent survey showed six main findings as follows: (1) a four-fold increase in the estimated number of clinically definite patients with MS in 2003 (9900; crude MS prevalence, 7.7/100,000) compared with 1972; (2) a shift in the peak age at onset from early 30s in 1989 to early 20s in 2003; (3) a successive proportional decrease in optic-spinal involvement in clinically definite patients with MS; (4) a significant north–south gradient for the CMS/OSMS ratio; (5) after subdivision of the mainland (30–45° North) into northern and southern parts at 37°N, northern-born northern residents (northern patients) showed a significantly higher CMS/OSMS ratio and higher frequency of brain lesions fulfilling the Barkhof criteria (Barkhof brain lesions) than southern-born southern residents (southern patients); (6) among northern patients, the absolute numbers of patients with CMS and those with Barkhof brain lesions rapidly increased with advancing birth year.
Conclusions These findings suggest that MS phenotypes are drastically altered by environmental factors, such as latitude and "Westernization." Multiple Sclerosis 2009; 15: 159–173. http://msj.sagepub.com

Key words: epidemiology; Japanese; latitude; magnetic resonance imaging; multiple sclerosis; optic-spinal

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that results from a complex interplay between genetic and environmental factors [1]. MS is rare in Asians, but when it does occur, selective and severe involvement of the optic nerve and spinal cord is characteristic [2]. In 1958, Okinaka, et al. (1958) first reported a series of Japanese patients

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Received 14 December 2007; revised 8 May 2008; 9 June 2008; 19 July 2008; 19 August 2008; accepted 4 September 2008

 10.1177/1352458508098372

with demyelinating diseases, among whom 175 of 270 cases were described as having Devic's neuromyelitis optica (NMO), and classical MS was rare. These early researchers also found intermediate cases between NMO and classical MS [3,4]. Thereafter, only monophasic NMO has been referred to as NMO, while relapsing NMO is included within the spectrum of MS. In Japan, the latter group has been designated as having the optic-spinal MS (OSMS) and its clinical criteria, proposed in 1996 [5], have frequently been used in clinical research on Japanese patients. Recently, NMO-IgG, a newly identified marker for NMO [6], was also detected in a fraction of Japanese patients with OSMS [7,8], and OSMS is now postulated to be the same disease as relapsing NMO [9]. However, further studies are required to clarify whether MS, OSMS, and NMO are distinct diseases or whether they form a continuum [8–11].

NMO is also a major demyelinating disease in Africans [12,13]. Differences in phenotypes among races are assumed to result from genetic differences [14]. However, studies on migrants have shown changes in not only the prevalence but also the phenotype of MS, which are attributable to the early-life environment [15–21]. Asian and African descendents in the United Kingdom [17,18] and returning migrants from France to the French West Indies [21] are behind the emergence of classical MS in place of NMO in these populations, whereas Caucasian descendents in tropical Colombia show more frequent optic-spinal involvement [20]. Although these migration studies indicate the influence of exogenous factors on MS susceptibilities and phenotypes, such interpretations must be made with caution because the admixture of genes could be equally influential [14], as observed in a genetic study on Mexican mestizos, which showed that patients who presented with classical MS also harbored more Caucasian genes [22].

An alternative method for determining whether genetic or environmental factors are responsible for the manifestation of demyelinating diseases is to investigate phenotypic changes over time in genetically homogeneous and geographically isolated populations that have experienced rapid environmental changes. Japanese, for whom interracial marriage with Caucasians remains exceptional, are suitable for such a study. Because nationwide surveys of MS in Japan were conducted using essentially identical criteria in 1972, 1982, and 1989 [23,24], we decided to perform a fourth nationwide survey in 2004 to uncover any phenotypic changes in MS that have occurred during Japan's period of "Westernization."

Methods

Survey procedures

The fourth nationwide survey of MS was conducted by the Research Committees of Neuroimmunological Diseases and of Epidemiology of Intractable Diseases, sponsored by the Ministry of Health, Labour and Welfare, Japan. The study was approved by the Kyushu University Ethics Committee. The survey was undertaken in two steps. First, a preliminary survey was undertaken to ascertain the approximate number of patients with MS in Japan, and second, a survey was conducted using a questionnaire sheet for each patient. The hospitals included in the study were randomly selected from the directory of all registered hospitals throughout Japan. Selection was made according to a stratification based on the number of beds in each hospital, in which increasing numbers of beds led to increasing probabilities of being selected [25]. The sampling rates were approximately 8%, 13%, 24%, 43%, 83%, and 100% for the strata of general hospitals with 20–99 beds, 100–199 beds, 200–299 beds, 300–399 beds, 400–499 beds, and 500+ beds, respectively. All university hospitals and as well as those in which council members of the Japanese Society of Neurology and members of the Committees of Medical Facilities for Children and the Japanese Society of Child Neurology were working were also surveyed.

The questionnaire for the preliminary survey on patients with MS who visited hospitals due to disease within the period from January 1 to December 31, 2003 was mailed to 6708 departments (comprising 1933 neurology/internal medicine, 1227 orthopedics, 997 psychiatry, 945 pediatrics, 831 ophthalmology, 759 neurosurgery, and 16 rehabilitation departments) together with the diagnostic criteria in January 2004. In Japan, all patients with MS, including monophasic NMO, are requested to visit hospitals at least once every year for registration of intractable diseases with the government to be subsidized for their medical costs, which are not covered by health insurance. Following the collection and collation of the first questionnaire, a second questionnaire was forwarded to those institutions reporting patients in the first survey. The second questionnaire requested detailed clinical information on individual patients, including their ages at onset and examination, sex, birthplace, present address, symptoms based on history and signs from physical examination (Supplementary Table), laboratory findings, course, treatment, and prognosis. Patients reported by more than one hospital or department were treated as duplicates.

Supplementary Table Neurological symptoms or signs during the course of illness in clinically definite cases of multiple sclerosis

	Clinically definite MS[a]	
	1989 (n = 861)	2004 (n = 1493)
Mental impairment	20.4	17.4
Aphasia, apraxia, agnosia	5.1	4.1
Generalized convulsion	8.3	3.8
Visual loss	70.4	56.1
Optic atrophy	52.2	32.3
Visual field defect	33.4	27.8
Diplopia	28.4	21.3
Internuclear ophthalmoplegia	6.1	7.9
Nystagmus	36.5	27.1
Dysarthria	30.5	21.9
Dysphagia	17.7	10.4
Facial palsy	18.3	13.3
Quadriparesis	38.3	18.4
Paraparesis	48.3	43.4
Hemiparesis	37.5	35.5
Spasticity	55.9	47.6
Babinski reflex	64.1	58.7
Sensory disturbance		
Face	25.6	21.2
Segmental	36.8	34.5
Below a certain level	31.3	37.9
Hemi	33.8	33.7
Transverse myelitis	36.7	27.4
Recurrent	22.2	15.4
Limb ataxia	37.4	26.3
Truncal ataxia	33.5	30.5
Disturbance in urination	61.1	49.6
Painful tonic spasm	28.7	18.1
Lhermitte sign	32.5	29.7

[a]% of all cases for which information could be obtained regarding each of these items.

Diagnostic criteria

The diagnostic criteria used for the present survey were based on those used for the first nationwide survey in 1972 [23], except that the limitation of age at onset was removed, as it was in the third survey [24]. The criteria required multiplicity in time and space and were essentially the same as Schumacher's criteria [26]. Briefly, the criteria used for relapsing–remitting MS in the present survey consisted of three items for clinically definite MS: (1) symptoms and signs due to multifocal lesions in the CNS (more than two lesions in the CNS); (2) remissions and exacerbations (multiplicity in time); and (3) exclusion of other diseases, such as tumors, syphilis, cerebrovascular accident, cervical spondylosis, angiomas, subacute myelo-optico-neuropathy, neuro-Behçet, cerebellar degeneration, human T-lymphotropic virus-I-associated myelopathy/tropical spastic paraparesis, and collagen diseases. Clinically definite MS fulfilled all of the criteria, while a diagnosis of possible MS was made when all three criteria for clinically definite MS could not be fulfilled, but the signs were suggestive. The criteria for primary progressive MS (PPMS) were taken from McDonald's criteria [27]. Data from cases with monophasic NMO were also collected. The criteria for monophasic NMO were as follows: acute bilateral visual impairment (optic neuritis) and transverse myelitis occurring successively within several weeks.

Classification of clinical phenotypes

The classification of MS subtypes was solely based on the clinically estimated sites of the lesions. The second questionnaire requested the responders to check the clinically estimated sites of the lesions according to the symptomatology during the entire clinical course among the following sites: optic nerve, cerebrum, cerebellum, brainstem, and spinal cord. Moreover, the questionnaire also requested the responders to check for the presence of any of the signs and symptoms listed in Supplementary Table during the entire clinical course. The survey center classified each case into the following clinical subtypes based on the clinically estimated lesion sites reported by each institution: OSMS involving the optic nerve and the spinal cord; optic-brainstem-spinal MS (OBSMS) involving the optic nerve, brainstem, and spinal cord; brainstem-spinal MS (BSMS) involving the brainstem and the spinal cord; spinal MS (SMS) involving only the spinal cord, which was identical to recurrent myelitis without any known cause; and conventional MS (CMS), which involved multiple sites of the CNS, including the cerebrum or cerebellum. If there was no information on the lesion sites or the symptoms and signs during the entire course were incompatible with the lesion sites, the cases were placed into the unclassified category. As SMS is generally regarded as a limited form of OSMS [28], OSMS and SMS were grouped together and compared with other forms (CMS, OBSMS, and BSMS) that involved multiple sites of the CNS (such as the cerebrum, cerebellum, and brainstem) in some analyses. Because many intermediate cases between relapsing NMO and classical MS [3] have been reported in Japanese, the term "relapsing NMO" was not used in the four nationwide surveys. Thus, relapsing NMO was considered to be included in the OSMS subgroup in the present survey, according to the confined involvement of the optic nerve and the spinal cord. This was distinct from CMS presenting with multiple sites of CNS involvement including the cerebrum or cerebellum.

Statistical analysis

The estimated total number of patients with MS in Japan was calculated by summing the figures for the

total reported number of patients in each stratum divided by the ratio of responding institutions to the number of surveyed institutions in each stratum. The formulas used to estimate the total number of patients and the 95% confidence intervals (CIs) have been described in detail elsewhere [29,30]. The crude prevalence rate per 100,000 people was determined from the population of Japan in 2003. Statistical analyses of numerical variables were initially performed using the Kruskal–Wallis H test. When statistical significance was found, the Mann–Whitney U test was used to determine the statistical significance of differences among subgroups. Uncorrelated P values were multiplied by the number of comparisons (Bonferroni–Dunn's correction) to calculate corrected P values. Differences in the ratios between two groups were tested for significance by the $\cdot^2$ test or Fisher exact test when the criteria for the $\cdot^2$ test were not fulfilled. All statistical analyses were performed by three committee members (MO, TM, and KS).

Results

Comparisons with previous survey results

In the preliminary survey, 3749 institutions (55.9%) responded and reported 4827 patients with MS, including 849 patients with possible MS. In the second questionnaire, detailed data were collected for 1919 patients (39.3% of the preliminary survey), including 30 duplicate cases. There were no significant regional differences in the response rates. Specifically, there was no significant correlation between the response rate and the northern latitude of present residence in either the preliminary ($r = 0.067$, $P = 0.6522$) or second ($r = 0.048$, $P = 0.7431$) survey by Spearman's rank correlation test. Thus, the estimated number of clinically definite patients with MS in 2003 was 9900 (95% CI: 9100–10,700), representing a four-fold increase compared with the first nationwide survey in 1972 (Table 1) [23]. The estimated crude prevalence was 7.7/100,000 (95% CI: 7.1–8.4). The proportions of patients with clinically definite MS and female patients had increased since the first survey. The percentage of patients with monophasic NMO among all patients with MS as well as the absolute number of patients with this subtype had progressively decreased over time (Table 1; absolute numbers as follows: 82 in 1972, 77 in 1982, 46 in 1989, and 22 in 2004). Compared with the third nationwide survey, the peak age at onset had decreased and the second peak in the early 50s had disappeared (Figure 1A). Visual loss at onset and during the entire clinical course and optic atrophy during the entire course had decreased over the period of the four surveys. When the small numbers of patients with PPMS ($n = 40$) in the present survey were omitted, all statistical analyses gave essentially the same results as those described in the following sections (data not shown).

Clinical features and classification of patients with MS

Based on the clinically estimated sites of lesions, 1493 patients with clinically definite MS and completed questionnaires were classified as having CMS

Table 1 Comparison of demographic features among the four nationwide survey

Year of survey	1972	1982	1989	2004[a]
Estimated number of clinically definite patients with MS	2280	ND	3700	9900
Number of cases collated for the final survey	1084	1518	1270	1889
Sex ratio (male:female)	1:1.7	1:2.3	1:2.6	1:2.9
Clinically definite MS (%)[b]	46.9	55.9	67.8	84.5
Monophasic NMO (%)[b]	7.6	5.1	3.6	1.2
Age at onset (mean ± SD, years)[b]	33 ± 13	32 ± 13	34 ± 13	32 ± 13
Age at examination (mean ± SD, years)[b]	39 ± 13	40 ± 13	41 ± 14	42 ± 14
Mean disease duration (years)[b]	6	8	8	10
Familial occurrence (%)	1	1.3	ND	1.1
Visual loss at onset (%)	41.8	34.6	36.6	29.5
Visual loss during entire course (%)	79	ND	70.4	56.1
Optic atrophy during entire course (%)	62	ND	52.2	32.3
Quadriparesis during entire course (%)	ND	ND	38.3	18.4
Transverse myelitis during entire course (%)	ND	ND	36.7	27.4

MS, multiple sclerosis; ND, not determined; NMO, neuromyelitis optica.
[a]Interferon beta-1b, the only disease-modifying drug available in Japan since 2001, was administered in 37.2% of the cases in 2003.
[b]Data from all patients with MS including possible MS and monophasic NMO are shown. When the small numbers of primary progressive patients with MS ($n = 40$) in the present survey were omitted, all statistical analyses in the following sections gave essentially the same results.

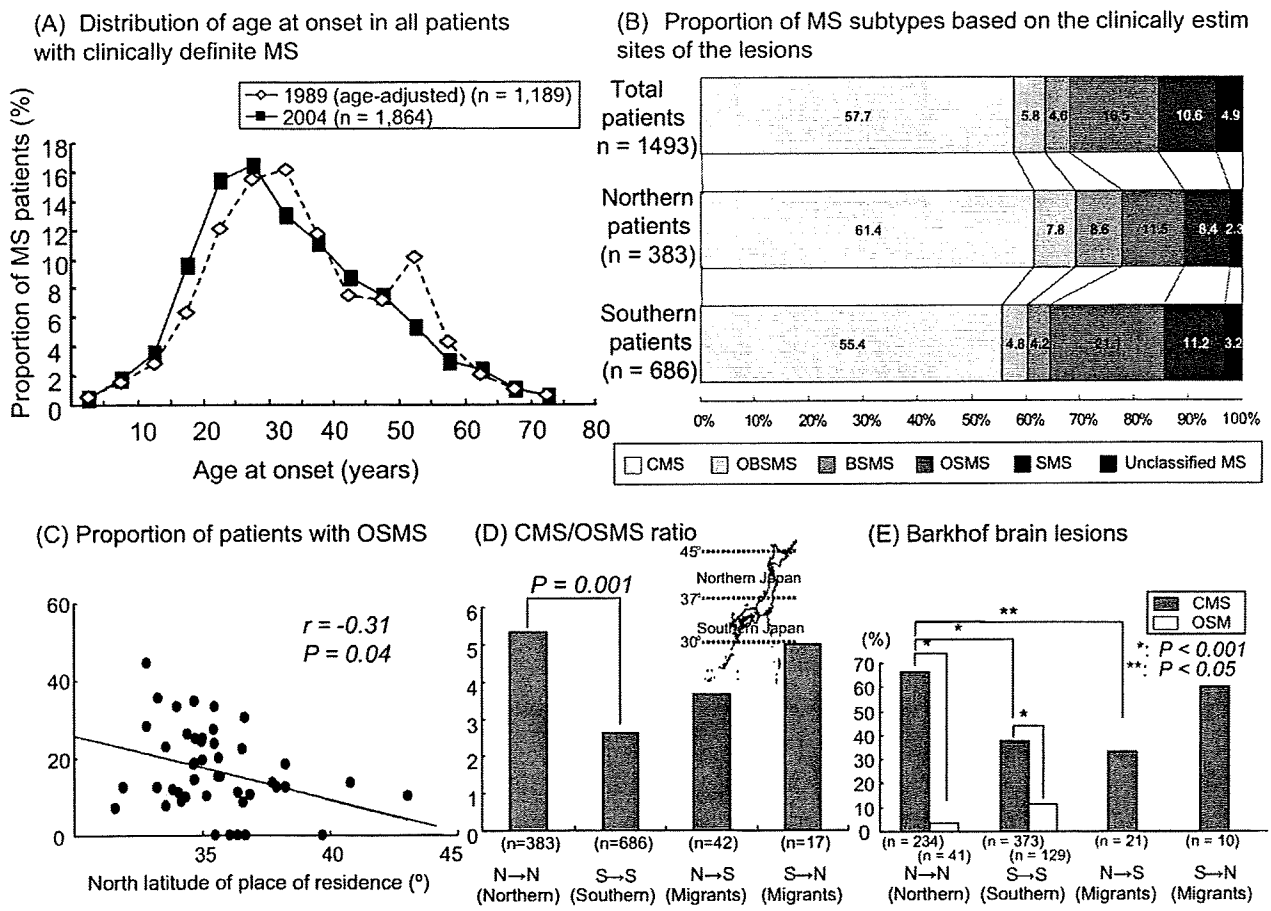

Figure 1 (A) Distribution of ages at onset in all patients with clinically definite MS. (B) Proportions of MS subtypes based on the clinically estimated sites of lesions. (C) Relationships between the OSMS percentages in the 46 prefectures of Japan and the northern latitudes of prefecture office locations. (D) CMS/OSMS ratios in relation to place of birth and residence. (E) Frequencies of brain magnetic resonance imaging lesions fulfilling the Barkhof criteria in relation to place of birth and residence. In (A), the proportions of patients with MS at each age at onset in 1989 have been adjusted to the age distribution of the Japanese population in 2003. Note that the age at onset curve shifts toward the younger side in 2004, whereas the second peak around the early 50s seen in 1989 is no longer evident. In (B), only those patients with known birthplace and present residence were analyzed. Here, the proportions of patients with MS showing the CMS, OBSMS, and BSMS phenotypes are higher among northern patients than among southern patients, whereas the OSMS and SMS phenotypes show the reverse trend. In (C), there is a significant positive correlation between the OSMS percentage and the latitude of the place of residence ($P < 0.05$). The same is true for the birthplace ($P < 0.05$) (data not shown). In (D), the Japanese mainland (inset in D), located from 30° North to 45° North, is arbitrarily divided into northern and southern parts at a latitude of 37° North. The respective CMS/OSMS ratios are shown for northern-born northern residents (N• N), southern-born southern residents (S• S), northern-born southern residents (N• S) and southern-born northern residents (S• N). The CMS/OSMS ratio is significantly higher in northern-born northern residents (northern patients) than in southern-born southern residents (southern patients) ($P < 0.001$). In (E), among patients with CMS, brain lesions fulfilling the Barkhof criteria are significantly more common in northern patients than in southern patients. Among patients with CMS, the frequency of Barkhof brain lesions is significantly lower in northern-born southern residents than in northern-born northern residents. The frequencies of Barkhof brain lesions in the OSMS groups are not shown in migrants because the sample numbers were too small. BSMS, brainstem-spinal form of multiple sclerosis; CMS, conventional form of multiple sclerosis; n, number of patients whose information was obtained; OBSMS, optic-brainstem-spinal form of multiple sclerosis; OSMS, optic-spinal form of multiple sclerosis; SMS, spinal form of multiple sclerosis.

(57.7%), OBSMS (5.8%), BSMS (4.6%), OSMS (16.5%), SMS (10.6%), or unclassified MS (4.9%) (Figure 1B). There were no significant differences in disease durations among the subtypes other than a significantly shorter disease duration in patients with SMS compared with patients with CMS and OSMS (Table 2). Comparisons of the clinical features between patients with CMS and OSMS showed significant differences in many aspects, similar to previous findings in Japanese patients [2]. Compared with patients with CMS, patients with OSMS showed a significantly higher

Table 2 Clinical characteristics among each multiple sclerosis subgroup classified according to the clinically estimated sites of the lesions

	OSMS (n = 246)	CMS (n = 862)	P value	SMS (n = 158)	BSMS (n = 68)	OBSMS (n = 86)
Sex ratio (male:female)	1:4.5	1:2.4	<0.001	1:2.3‡	1:3.5	1:4.4
Age at onset (years)	35.4 ± 12.9	29.3 ± 12.5	<0.001	38.3 ± 13.5†	31.6 ± 11.8	31.1 ± 11.7‡
Age at examination (years)	47.1 ± 14.1	39.9 ± 13.6	<0.001	45.3 ± 13.5†	41.1 ± 11.8‡	41.8 ± 12.7‡
Disease duration (years)	11.7 ± 9.1	10.6 ± 8.4	NS	7.0 ± 6.4*,†	9.5 ± 6.9	10.9 ± 7.3
EDSS scores	4.3 ± 2.7	3.5 ± 2.9	<0.001	3.4 ± 2.3‡	2.6 ± 2.3*	4.0 ± 2.8
Symptoms during entire course						
Bilateral visual loss	131/246 (53.3%)	260/851 (30.6%)	<0.001	0/158 (0.0%)*,†	0/68 (0.0%)*,†	46/84 (54.8%)†
Transverse myelitis	113/231 (48.9%)	170/823 (20.7%)	<0.001	52/152 (34.2%)†,‡	24/68 (35.3%)§	24/82 (29.3%)‡
Paraparesis	139/239 (58.2%)	319/839 (38.0%)	<0.001	85/151 (56.3%)†	32/68 (47.1%)	41/83 (49.4%)
Quadriparesis	46/238 (19.3%)	160/848 (18.9%)	NS	20/151 (13.2%)	14/66 (21.2%)	19/83 (22.9%)
Sensory impairment below a certain level	141/224 (62.9%)	223/777 (28.7%)	<0.001	75/148 (50.7%)†	30/65 (46.2%)§	31/75 (41.3%)*
Sphincter disturbance	155/238 (65.1%)	393/848 (46.3%)	<0.001	75/154 (48.7%)*	35/68 (51.5%)	48/81 (59.3%)
Severe motor disability at the time of last examination[a]	57/231 (24.7%)	142/805 (17.6%)	0.017	15/148 (10.1%)*	8/63 (12.7%)	18/80 (22.5%)
Secondary progression	19/246 (7.7%)	131/861 (15.2%)	0.003	11/158 (7.0%)§	5/68 (7.4%)	9/86 (10.5%)
Cerebrospinal fluid findings						
Marked pleocytosis (• 50 WBC/mm^3) or neutrophilia (• 5 neutrophils/mm^3)	21/191 (11.0%)	51/730 (7.0%)	NS	11/134 (8.2%)	6/60 (10.0%)	3/65 (4.6%)
Increased IgG index	31/106 (29.2%)	240/397 (60.5%)	<0.001	35/68 (51.5%)‡	21/38 (55.3%)‡	22/47 (46.8%)
Brain MRI findings						
• 1 Gd-enhanced lesion or • 9 T2 brain lesions	41/226 (18.1%)	507/840 (60.4%)	<0.001	31/146 (21.2%)†	30/66 (45.5%)*	33/79 (41.8%)*,†
• 9 T2 brain lesions	37/226 (16.4%)	390/840 (46.4%)	<0.001	24/146 (16.4%)†	22/66 (33.3%)‡	31/79 (39.2%)*
• 1 Gd-enhanced lesion	10/199 (5.0%)	292/688 (42.4%)	<0.001	12/133 (9.0%)†	19/62 (30.6%)*	15/72 (20.8%)*,†
• 1 juxtacortical lesion	28/218 (12.8%)	303/786 (38.5%)	<0.001	11/138 (8.0%)†	9/66 (13.6%)†	17/78 (21.8%)§
• 3 periventricular lesions	59/221 (26.7%)	526/806 (65.3%)	<0.001	34/143 (23.8%)†	27/66 (40.9%)†	37/80 (46.3%)*,†
• 1 infratentorial lesion	42/219 (19.2%)	539/827 (65.2%)	<0.001	27/145 (18.6%)†	45/67 (67.2%)*	57/81 (70.4%)*
Lesions fulfilling the Barkhof criteria	19/223 (8.5%)	382/844 (45.3%)	<0.001	16/145 (11.0%)†	18/67 (26.9%)*,§	25/81 (30.9%)*
No cranial lesion	94/223 (42.2%)	16/844 (1.9%)	<0.001	68/145 (46.9%)†	7/67 (10.4%)*,†	8/81 (9.9%)*,†
Spinal cord MRI findings						
• 1 T2 lesion	203/223 (91.0%)	508/724 (70.2%)	<0.001	145/153 (94.8%)†	60/66 (90.9%)†	69/74 (93.2%)†
LESCL	93/223 (41.7%)	121/724 (16.7%)	<0.001	46/153 (30.1%)†	15/66 (22.7%)‡	24/74 (32.4%)†
Gd-enhanced lesion	99/181 (54.7%)	187/653 (28.6%)	<0.001	74/127 (58.3%)†	37/63 (58.7%)†	29/67 (43.3%)

BSMS, brainstem form of multiple sclerosis; CMS, conventional form of multiple sclerosis; EDSS, expanded disability status scale of Kurtzke; Gd, gadolinium; LESCLs, longitudinally extensive spinal cord lesions extending 3 or more vertebral segments; MRI, magnetic resonance imaging; NS, not significant; OSMS, optic-spinal multiple sclerosis; OBSMS, optic-brainstem-spinal multiple sclerosis; SMS, spinal form multiple sclerosis (recurrent myelitis of unknown cause).

[a]Chair-bound or worse.

*P < 0.01 (vs OSMS), correlated P values multiplied by the number of comparisons (6 times).

†P < 0.01 (vs CMS).

‡0.01 • P < 0.05 (vs OSMS).

§0.01 • P < 0.05 (vs CMS).

age at onset, greater proportion of women, higher expanded disability status scale (EDSS) of Kurtzke score [31], and higher frequencies of bilateral visual loss, transverse myelitis, paraparesis, sensory impairment below a certain level, and sphincter disturbance. By contrast, patients with OSMS had significantly lower frequencies of secondary progression and increased IgG index in the cerebrospinal fluid (CSF) than patients with CMS. The occurrences of brain lesions fulfilling the Barkhof criteria [32] (Barkhof brain lesions) and each item of the criteria were significantly higher in patients with CMS than in patients with OSMS ($P < 0.001$), whereas longitudinally extensive spinal cord lesions (LESCLs) extending across three or more vertebral segments and gadolinium-enhanced spinal cord lesions showed the reverse trend ($P < 0.001$) (Table 2).

Regarding the other subtypes classified on the basis of the clinically estimated lesion sites, although the disease durations did not differ significantly among patients with OSMS, OBSMS, and BSMS, patients with OBSMS and BSMS showed lower frequencies of transverse myelitis and sensory levels, higher frequencies of brain magnetic resonance imaging (MRI) lesions and less frequent occurrence of LESCLs than patients with OSMS (Table 2). Patients with OBSMS and BSMS showed some similarities to patients with CMS due to their higher frequencies of brain lesions, although patients with BSMS had lower EDSS scores and patients with OBSMS had more frequent bilateral visual impairment and LESCLs than patients with CMS. By contrast, patients with SMS were similar to patients with OSMS in many aspects, including higher age at onset, fewer brain MRI lesions, and higher frequency of LESCLs, although the severity of the spinal cord lesions represented by the EDSS scores was milder and an increased IgG index was more frequently observed in patients with SMS than in patients with OSMS, suggesting the possibility that SMS is, for the most part, an incomplete form of OSMS during the early course of disease. Therefore, in the following sections, the comparisons between patients with CMS and OSMS by geography and year of birth are supplemented by additional analyses in which OSMS and SMS patients were combined into one group (27.1% of all patients with MS) and patients with CMS, BSMS, and OBSMS were combined into another group (68.1% of all patients with MS).

Differences in clinical phenotypes by latitude and year of birth

A significant negative correlation was found between the proportion of patients with OSMS and the northern latitude of present residence ($r = \cdot 0.31$, $P = 0.04$) (Figure 1C). When the mainland (30–45° North) was subdivided into northern and southern parts at approximately the midpoint (37° North), northern-born northern residents (defined as northern patients) showed a significantly higher CMS/OSMS ratio than southern-born southern residents (southern patients) (Figure 1D). Both the patients with OBSMS and BSMS showed some similarities to patients with CMS in terms of their clinical features, and these forms of the disease were twice as common in northern patients than in southern patients, whereas SMS with similar demographic features to OSMS was more common in southern patients than in northern patients (Figure 1B). Thus, the CMS+OBSMS+BSMS/OSMS+SMS ratio was also significantly higher in northern patients than in southern patients (data not shown). Migrants, especially northern-born southern residents, showed intermediate CMS/OSMS ratios between those of the northern and southern patients (Figure 1D).

The CMS/OSMS ratio increased dramatically with advancing year of birth among northern patients, but such a trend was more modest among southern patients (Figure 2A, B). There was a steady increase in the absolute numbers of patients with CMS among both northern and southern patients with advancing year of birth, whereas the numbers of patients with OSMS showed a modest tendency to decrease with advancing year of birth among northern patients, but had no definite trend among southern patients.

Differences in MRI findings by latitude and year of birth

Among the patients with CMS, northern patients showed a significantly higher frequency of Barkhof brain lesions than southern patients ($P < 0.001$) and northern-born southern residents ($P = 0.02$) (Figure 1E), whereas southern patients had significantly more LESCLs than northern patients ($P < 0.001$) (Supplementary Figure 1A). When the CMS+OBSMS+BSMS and OSMS+SMS groups were analyzed, essentially the same results were obtained (data not shown). The proportions and absolute numbers of patients with Barkhof brain lesions steadily increased with advancing year of birth among both northern and southern patients, but the trend was far more marked in northern patients (Figure 2C, D). The proportions of patients with LESCLs successively decreased with advancing year of birth among both northern and southern patients, whereas the absolute numbers remained largely unchanged over the wide range of birth

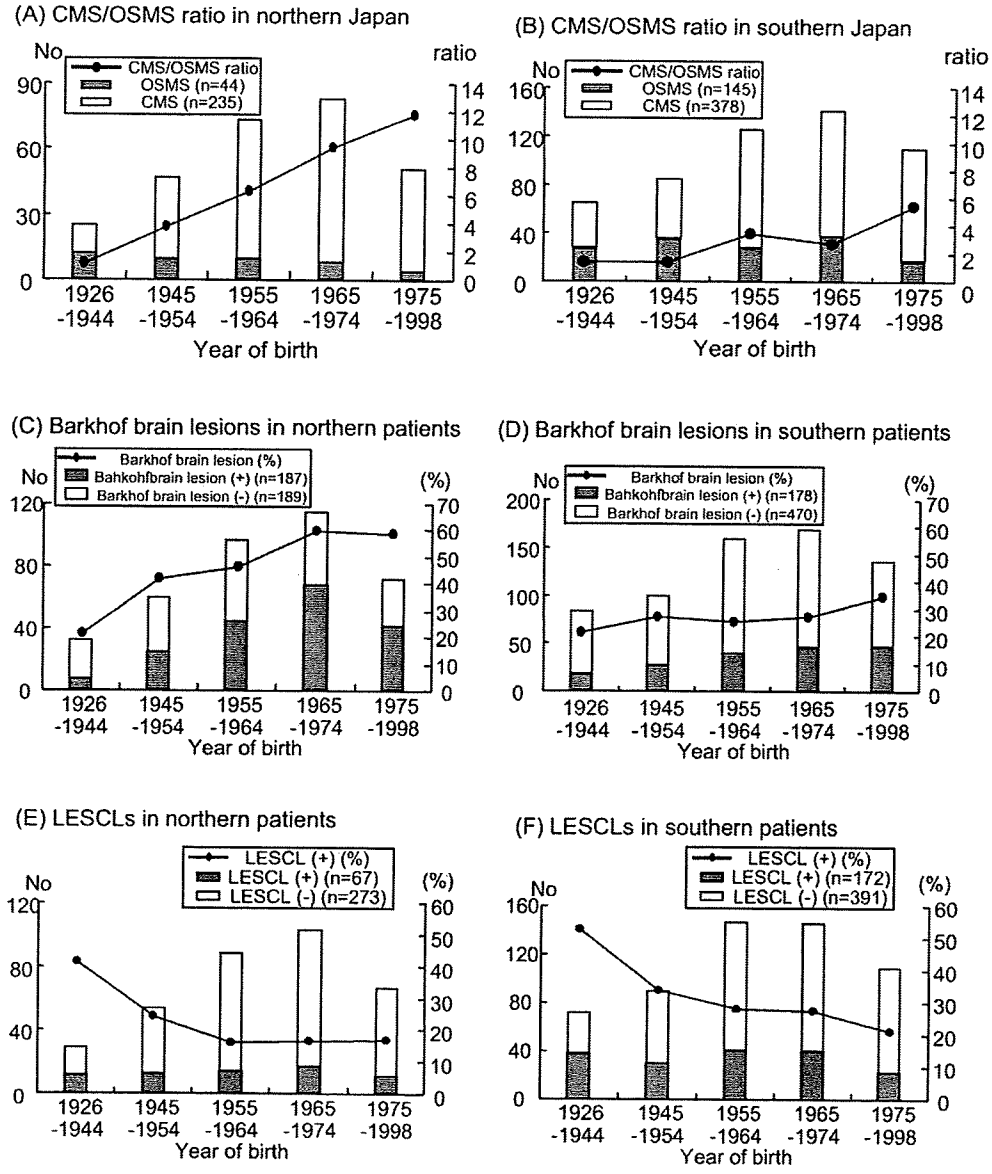

Figure 2 Changes in the clinical phenotypes of patients with clinically definite MS in relation to year of birth. CMS/OSMS ratios and absolute numbers of patients with each phenotype in relation to year of birth among northern (A) and southern (B) patients. Changes in the proportions and absolute numbers of patients with Barkhof brain lesions in relation to year of birth among northern (C) and southern (D) patients. Proportions and absolute numbers of patients with LESCLs in relation to year of birth among northern (E) and southern (F) patients. In (A) and (B), the CMS/OSMS ratios are compared between northern and southern patients and configured according to the year of birth. The ratio steadily increases with advancing year of birth. Each bar indicates the absolute number in each group in the indicated birth years while each dotted line shows the changes in the ratios or percentages. In (C-F), the proportions and absolute numbers of patients with brain lesions fulfilling the Barkhof criteria are increased among northern patients with descending year of birth, while only the proportions of patients with LESCLs decrease with descending year of birth among both northern and southern patients. Bars indicate the absolute numbers in each group with the indicated birth years, while dotted lines show the changes in the positive percentages of the indicated groups. BSMS, brainstem-spinal multiple sclerosis; CMS, conventional multiple sclerosis; LESCLs, longitudinally extensive spinal cord lesions extending for three or more vertebral segments on MRI; n, number of patients whose information was obtained; OBSMS, optic-brainstem-spinal multiple sclerosis; OSMS, optic-spinal multiple sclerosis; SMS, spinal multiple sclerosis.

years (Figure 2E, F). The frequency of Barkhof brain lesions was significantly higher in patients born after 1955 than in those born before 1954 among northern patients ($P < 0.001$), but not among southern patients. The frequencies of patients with LESCLs were significantly higher among patients born before 1954 than among those born after 1955 ($P < 0.001$).

Differences in the distributions of MS subtypes by latitude and year of birth

The proportions and absolute numbers of patients with CMS with Barkhof brain lesions, a classical form of Western-type MS, increased steadily with advancing year of birth in both northern and southern patients, but these increases were more pronounced in northern patients than in southern patients (Supplementary Figure 1B, C). The proportions of OSMS+SMS patients with LESCLs, representing a prototypic form of Asian-type MS, decreased among recently born patients in both the northern and southern groups, but decreases in absolute numbers were only modestly seen in those born after 1955 and only in the northern group (Supplementary Figure 1D, E). On the other hand, the absolute numbers of OSMS+SMS patients without LESCLs increased among northern patients born after 1955 (Supplementary Figure 1D). The same was also true for patients with OSMS (data not shown). By contrast, there was a trend toward increases in the proportion and absolute number of patients with LESCLs with advancing year of birth among northern patients with CMS, but the increase in absolute number was minimal among southern patients with CMS (Supplementary Figure 1F, G).

Multiple logistic analyses

Multiple logistic analyses showed that CMS phenotype ($P < 0.0001$), northern residence ($P < 0.0001$), increased CSF IgG index ($P = 0.0064$), and EDSS score ($P = 0.0380$) had significant positive associations with Barkhof brain lesions, whereas marked CSF pleocytosis was negatively associated with these measures ($P = 0.0026$) (Table 3). By contrast, EDSS score ($P < 0.0001$), marked CSF pleocytosis ($P = 0.0007$), OSMS ($P = 0.0007$), and disease duration ($P = 0.0284$) were positively associated with LESCLs, whereas increased IgG index was negatively associated ($P = 0.0398$) (Table 4). In analyses for either Barkhof brain lesions or LESCLs, substitution of CMS with CMS+OBSMS+BSMS, OSMS with OSMS+SMS, northern birth with northern residence, and CSF oligoclonal bands with increased IgG index gave essentially the same results (data not shown).

Discussion

The present study had some limitations because the questionnaires were answered by many different clinicians across the country, and the response rate in the second survey was not high. Concerning the relatively low response rate to this type of nationwide epidemiological survey in Japan, the assumption that the mean number of patients among responding hospitals is equal to that among non-responding hospitals has already been validated [29]. Moreover, there were no significant regional differences in the response rates in the present study and no significant differences between the northern and southern response rates (59% vs 55% in the preliminary survey and 46% vs 39% in the second survey, $P > 0.1$). Differences in the ascertainment rates between the northern and southern parts of Japan in previous surveys, undertaken 15 to 30 years ago, was unclear; however, as the previous surveys were carried out using practically the same methodology as the present one, it is unlikely that there were large ascertainment differences between northern and southern parts of Japan in those surveys. Therefore, we consider that our results would not be seriously distorted by the relatively low response rates. In addition, 88% of the questionnaires were collected from neurologists, 70% of whom had previously participated in a randomized controlled trial of interferon beta-1b [33], which increases the quality of the data on one hand, but produces a selection bias on the other hand. However, the validity of the data is indirectly supported by two aspects. First, although the present figure derived from clinical symptomatology-based criteria could be an underestimate compared with estimates based on recent MRI-based diagnostic criteria [27], the estimated number of patients with MS is close to the number of patients with MS (10,391) registered in the government's health care system for intractable diseases in 2003. Second, the increased MS prevalence revealed by the present study is concordant with the results of two recent epidemiological surveys carried out in the northernmost island of Japan, namely 8.57/100,000 [34] and 10.2/100,000 [35]. Both studies showed a four-fold increase in MS prevalence over a 30-year period and one of them also disclosed a similar frequency of OSMS (16% at 42° North) to that found in the present survey [34].

The steady rise in the prevalence of clinically definite MS can be explained by the increased availability of MRI, which helps with the exclusion of other diseases, and the increased number of practicing neurologists. Increased survival due to improved case ascertainment could be inferred from the prolongation of mean disease duration shown in more recent surveys. Thus, the increase in MS prevalence appears to be partly attributable to improved case ascertainment. However, the younger age at onset and two-fold increase in the proportion of females, which corresponds to a worldwide increase in the number of female patients with MS [36–38], in the latest survey, cannot be fully explained by improved case ascertainment. Orton, et al. [39] reported that year of birth is a significant predictor for sex ratio; the