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Genetic and immunologic analysis on moya-moya

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SUMMARY The genetic and immunologic abnormalities associated with the pathogenesis of moya-moya were assessed in 23, 13 children and 10 adults with angiographically diagnosed moya-moya. In HLA-A, -B, -C stereotyping, an association was found of AW24, BW46, and BW54 with relative risks of 3·83, 6·50, and 3·58 respectively. Natural T cell toxic autoantibody was detected by FACS analysis in sera from five out of 23 patients. Millipore filter assay for autoantibody against double-stranded DNA revealed higher than normal binding in sera from four out of 18 patients. Anti-vessel antibody which might be responsible for vascular change associated with moya-moya was not detected in any of the 23 patients studied. Significant association of the disease with certain HLA types, in addition to the presence of natural T cell toxic autoantibody and anti-double-stranded DNA antibody in patients' sera, supports the theory that genetic and immunologic disturbances may underly the pathogenesis of moya-moya.

Since moya-moya was reported initially by Kudo *et al* in 1956,[1] numerous studies have been carried out concerning the symptoms, neuroradiology, epidemilogy, statistics, etc of the disorder. However, many questions remained unanswered. Among these are the important questions of whether moya-moya is a single clinical entity and what is the underlying pathogenesis. At the present time, the cause of this disease process is unknown.

Previously we reported on the occurrence in siblings, including six patients in three families among 49 cases of moya-moya. This strongly suggests a hereditary factor.[2] The present study attempted to elucide further the pathogenic mechanism of moya-moya. Patients clinically and angiographically diagnosed as moya-moya were assessed genetically and immunologically by tests for human leukocyte antigen (HLA), natural T cell toxic autoantibody (NTA), anti-double-stranded DNA antibody (anti-ds DNA antibody), and anti-vessel antibody.

Material and methods

Patients The 23 patients (five male and 18 female) included in this study were confirmed by angiography to have moya-moya. Clinical information on each patient concerning age of onset, clinical presentation (transient ischaemic attack, infarction, epilepsy, intracranial haemorrhage, or headache), follow-up results (good, fair, or poor) and surgical treatment are shown in table 1.

HLA typing HLA-A, -B, and -C stereotyping of peripheral blood lymphocytes from moya-moya patients was kindly performed by Dr T Juji, Blood Tranfusion Service, Tokyo University Hospital, Tokyo, Japan.

Natural T Cell Toxic Autoantibody Peripheral blood lymphocytes were obtained from whole heparinised blood of a normal volunteer by centrifugation over a Ficol-Hypaque gradient. In order to remove B cells, the lymphocytes were incubated on Petri dishes that were precoated with rabbit anti-human immunoglobins. Non-adherent cells were used as target T cells for the detection of NTA. The cells were incubated with serum from each patient at 4°C for 30 min and then washed. They were stained with fluorescein isocyanate-labelled goat anti-human immunoglobulins and analysed by the fluorescence activated cell sorter (FACS, Becton Dikinson Electronics Laboratory, Mountain View, Calif.). Analysis of fluorescence profiles of stained cells was performed according to the method described by Herzenberg *et al.*[3] As a control, T cells stained with normal serum and the same fluoresceinated reagent were analysed under identical conditions. The analytical patterns were recorded by direct photography of the oscilloscope.

Anti-double-stranded DNA Antibody (Anti-ds DNA Antibody) Analysis of anti-ds DNA antibody was generously carried out by Dr R Yokohari and Dr S Aotsuka, Division of Immunology, Clinical Research Institute, National Center Hospital, Tokyo, Japan, with a modification of the millipore filter method of Ginsberg and Keisel.[4]

Anti-vessel antibody In order to test for anti-vessel anti-

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Table 1 *Data of 23 moya-moya patients*

Case No	Sex	Age (yr)	Age of onset (yr)	Symptom or state of onset	Stage	Follow-up results	Surgical treatment	NTA	*Anti-ds-DNA Antibody (% binding)	Anti-Vessel Antibody	HLA Typing A	B	C
1	f	6	1	Infarction	Active	Poor	(−)	(++)	9·9	(−)	W24,(−)	W54,W60	W1,W3
2	f	5	3	TIA	Active	Fair	EMS	(−)	3·7	(−)	W24,(−)	W60,W61	—
3	f	5	5	TIA	Active	Good	(−)	(−)	—	(−)	—	—	—
4	f	15	9	TIA	Stationary	Good	(−)	(−)	1·7	(−)	—	—	—
5	f	17	10	TIA	Stationary	Good	(−)	(−)	0·2	(−)	—	—	—
6	f	19	9	TIA	Stationary	Good	(−)	(++)	2·8	(−)	—	—	—
7	f	10	2	TIA	Active	Fair	(−)	(−)	2·6	(−)	2,W24	W51,W52	—
8	f	13	4	TIA	Stationary	Good	(−)	(−)	2·6	(−)	W24,W33	7,W44	W7,(−)
9	f	13	7	TIA	Stationary	Poor	(−)	(−)	1·3	(−)	2, 26	W54,W60	W3,(−)
10	f	9	4	TIA	Stationary	Fair	EMS	(−)	0·6	(−)	W24,(−)	W51,W52	—
11	f	11	7	Epilepsy	Stationary	Good	(−)	(+++)	—	(−)	W24, 31	W51,W52	W3,(−)
12	f	16	9	TIA	Stationary	Good	(−)	(−)	—	(−)	—	—	—
13	f	21	6	Infarction	Stationary	Fair	(−)	(−)	—	(−)	2,W24	W46,W52	W1,W3
14	f	4	1	Infarction	Stationary	Fair	(−)	(−)	2·2	(−)	1,W24	W51,W61	W3,(−)
15	f	18	10	ICH	Stationary	Good	V-P shunt	(−)	0·2	(−)	11, 26	W54,(−)	W1,(−)
16	f	33	19	TIA	Stationary	Good	(−)	(−)	11·2	(−)	2,W33	W44,W61	W3,(−)
17	f	25	18	Headache	Stationary	Poor	DC	(−)	1·5	(−)	2,W24	7,W54	W1,(−)
18	f	54	52	ICH	Stationary	Good	(−)	(−)	0·0	(−)	1,W24	W35,W60	W3,(−)
19	m	44	31	TIA	Stationary	Fair	(−)	(−)	9·7	(−)	2,W24	W46,W52	W3,(−)
20	m	43	37	ICH	Stationary	Good	(−)	(−)	2·1	(−)	—	—	—
21	m	57	46	ICH	Stationary	Good	EMS, STA-MCA	(−)	13·6	(−)	11,W24	7,W54	W1,(−)
22	m	55	51	ICH	Stationary	Good	(−)	(+)	—	(−)	W24,(−)	W52,W59	W1,(−)
23	m	47	16	Epilepsy	Stationary	Good	(−)	(+)	0·2	(−)	W24,(−)	7,(−)	W3,(−)

TIA; transient ischaemic attack
ICH; intracranial haemorrhage
EMS; encephalo-myosynangiosis
DC; decompressive craniectomy
STA-MCA; STA-MCA anastomosis
NTA; natural T cell toxic auto-antibody
*; normal range: less than 5·37
Good; able to work
Fair; capable of self-care
Poor; incapable of self-care

Table 2 *Phenotype frequency of HLA-A, B and C in moya-moya*

Antigen	Our cases n = 18		Reviewed cases* n = 31		Total n = 49		Control n = 106	
	No	(%)	No	(%)	No	(%)	No	(%)
HLA-A1	1	(5·5)	0		1	(2·0)	2	(1·9)
A2	7	(38·9)	10	(32·2)	17	(34·7)	51	(48·1)
A11	2	(11·1)	2	(6·5)	4	(8·2)	15	(14·2)
AW24	†15	(83·3)	23	(74·2)	38	(77·6)	60	(56·6)
A26	3	(16·7)	8	(25·8)	11	(22·4)	22	(20·8)
AW31	0		7	(22·6)	7	(14·3)	18	(17·0)
AW32	1	(5·5)	1	(3·2)	2	(4·1)	0	(0)
AW33	2	(11·1)	5	(16·1)	7	(14·3)	14	(13·2)
HLA-B5	1	(5·5)	7	(22·6)	8	(16·3)	40	(37·7)
B7	4	(22·2)	2	(6·5)	6	(12·2)	12	(11·3)
B16	0		6	(19·4)	6	(12·2)	19	(17·9)
BW16	0		4	(12·9)	4	(8·2)	10	(9·4)
BW35	1	(5·5)	2	(6·5)	3	(6·1)	14	(13·2)
BW44	2	(11·1)	6	(19·4)	8	(16·3)	12	(11·3)
BW46	‡ 2	(11·1)	0		2	(4·1)	2	(1·9)
BW51	4	(22·2)	0		4	(8·2)	19	(17·9)
BW52	6	(33·3)	0		6	(12·2)	21	(19·8)
BW54	§ 6	(33·3)	11	(35·5)	‖17	(34·7)	13	(12·3)
BW60	4	(22·2)						
BW61 [BW40]	3	(16·7)	‖17	(54·8)	‖24	(49·0)	[36]	(34·0)
HLA-CW1	7	(38·9)					35	(33·0)
CW3	9	(50·0)					47	(44·3)
CW7	1	(5·5)					13	(12·3)

*reviewed cases in reference 7 and 10
†relative risk 3·83, $P < 0·05$
‡relative risk 6·50, $P < 0·05$
§relative risk 3·58 $P < 0·025$
‖relative risk 3·80, $P < 0·005$

body, indirect immunofluorescence was applied. A cortical artery of the brain, obtained from a temporal lobectomy, was used. A three micron thick section of the artery was put on a glass slide and fixed by acetone. The patient serum was incubated with the tissue section and the slide washed. The section then was overlaid with fluorescein conjugated goat anti-human immunoglobins. After washing, the slides were examined under a fluorescent microscope.

Results

HLA TYPING

HLA-A, -B, -C typing was performed in 18 patients. These results, in addition to those of 106 normal controls and those previously reported from 31 moya-moya patients,[5,6] are presented in tables 1 and 2. The relative risk (RR) of each HLA type in moya-moya patients was calculated by the following formula.

$$RR = \frac{\text{number of patients with corresponding type} \times \text{number of controls without corresponding type}}{\text{number of patients without corresponding type} \times \text{number of controls with corresponding type}}$$

Each relative risk of AW24, BW46, and BW54 was high, and statistically significant, that is, 3·83, 6·50, and 3·58 respectively.

NATURAL T CELL TOXIC AUTOANTIBODY

Sera from 23 patients were tested and five patients were revealed possessing NTA (table 1). None of these five patients had any previous medical history such as undergoing blood transfusion or pregnancy which might be associated with a "false positive" in the examination. In tests of sera from 101 normal donors, this was not demonstrated. The FACS profiles of patient 11 who possessed the highest titre is depicted in fig 1 in contrast with a normal control.

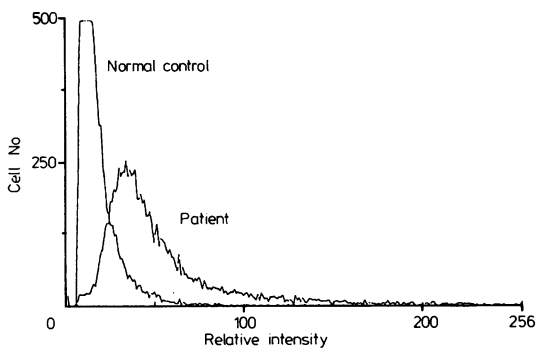

Fig 1 *FACS profile of human lymphocytes stained with serum from patient 11 and a normal subject.*

ANTI-DOUBLE-STRANDED DNA ANTIBODY

Analysis was performed with sera from 19 patients. As a control, sera from 15 normal volunteers were examined for anti-ds DNA antibody. The average of ds DNA bound by sera from normal subjects was 2·50 ± 1·43% (mean value and standard deviation). Therefore, the normal range was below 5·37%. The average value of moya-moya patients was 3·66 ± 4·14%, and this is significantly different from the normal. However, in four patients, the percentage of ds DNA bound was much higher than the normal range (fig 2). In the same analysis, anti-ds DNA antibody was specifically or preferentially demonstrated in sera from patients with lupus erythematosus among various autoimmune diseases, as shown in fig 2.

ANTIVESSEL ANTIBODY

By immunofluorescence, antibody blood vessels was not detected in sera from any of 23 patients (table 1).

RELATION BETWEEN CLINICAL AND GENETIC OR IMMUNOLOGIC FINDINGS

In patients with NTA or anti-ds DNA antibody or both, no correlation with the clinical findings was determined. Of the patients with such antibodies most were adults. Case 1, in which the clinical stage was "active" and the follow-up results were "poor", was remarkable in that both natural T cells antibody anti-ds DNA antibody were detected. Furthermore, this patient was typed as HLA-AW24 and -BW54 which overall showed a significant association with moya-moya.

Discussion

The major histocompatibility complex of man controls the human histocompatibility antigen expressed on leukocytes, that is, HLA and is inherited in a Mendelian fashion. Certain HLA types are reported to occur with high frequency in association with certain diseases. In our study of 18 patients, moyamoya was associated with HLA-AW24 (RR;3·83), BW46 (RR;6·50), and -BW54 (RR;3·58) (table 2). Particularly, the association with BW54 was also noted in 31 cases which have been already reported.[5,6] Moreover, when the pathogenesis of moya-moya is considered, it is notable that rheumatoid arthritis in Japanese patients, especially associated with vasculitis, showed a high relative risk associated with BW54.[7] This significant association between moya-moya and certain HLA types is consistent with the hypothesis that this disease may have, in part, a hereditary basis. In order to confirm this, a more detailed

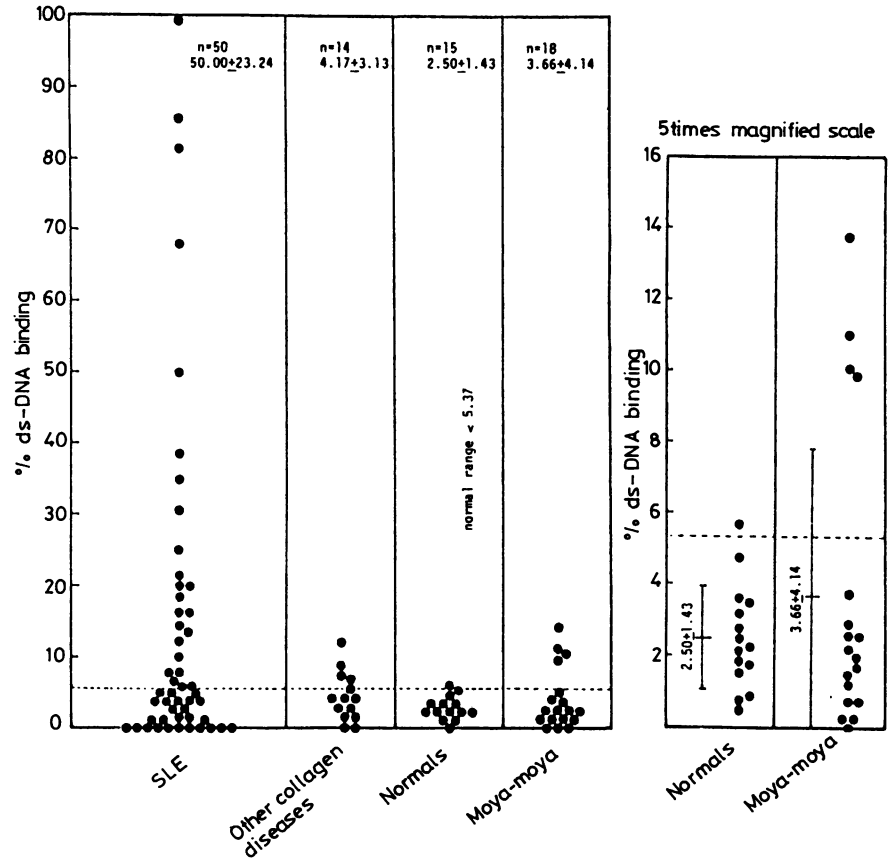

Fig 2 *Percentage double stranded DNA binding on millipore assay in various diseases.*

genetic study is required. We have therefore attempted to investigate the relation between moya-moya and HLA haplotype.

NTA was initially discovered in the New Zealand Black (NZB) mouse.[8] These mice spontaneously develop a systemic autoimmune disease similar to that of human systemic lupus erythematosus.[8] NTA also has been demonstrated in various human autoimmune diseases, such as systemic lupus erythematosis rheumatoid arthritis, progressive systemic sclerosis, myasthenia gravis, infectious mononucleosis.[9-11] Previously, Okumura *et al* had obtained the same results in several examinations of autoimmune diseases. The autoantibody was detected in about 45% of the patients with systemic lupus erythematosis, 31% with progressive systemic sclerosis, 23% with rheumatoid arthritis, and 86% with ulcerative colitis (table 3). Moreover, in the NZB mouse, NTA is associated with the selective loss of the suppressor T cell function, followed by the development of autoimmune disease.[12] Therefore, the frequency of NTA in moya-moya, compared to that in autoimmune diseases, suggests that an autoimmune mechanism may participate in the pathogenesis of this disease.

Table 3 *Incidence of natural T cell toxic autoantibody in various diseases*

	Incidence	% incidence
Normal	0/101	0
Systemic lupus erythematosus	46/102	45
Progressive systemic sclerosis	16/51	31
Rheumatoid arthritis	15/66	23
Behçet's disease	0/46	0
Aortitis syndrome	2/17	12
Ulcerative colitis	19/22	86
Moya-Moya	5/23	22

Antibodies against ds DNA are regarded as a characteristic of systemic lupus erythematosis and generally associated with the disease.[4] Moreover, accumulating evidence suggests that these antibodies play an important role in the pathogenesis of lupus nephritis.[4] The present study is the first in which anti-ds DNA antibody in moya-moya was

examined. The importance of this autoantibody in pathogenesis of moya-moya is not known at present. However, the fact cannot be ignored that this antibody was clearly demonstrated in four moya-moya patients.

The occlusive change in the terminal portion of the internal carotid artery, characteristic of moya-moya, might result from an immune reaction to the artery. The histopathology of this lesion resembles that of polyarteritis nodosa.[13] In our study, we failed to detect the existence of anti-vessel autoantibody in the sera of moya-moya, patients by the immunofluorescence method. The histopathological change in the internal carotid artery must be considered to be the most important matter in understanding moya-moya and warrants further investigation.

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