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Serum α_1-antitrypsin level and phenotype associated with familial moyamoya disease

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Abstract *Background:* Obstructive vascular lesions at the terminal portion of the internal carotid arteries are thought to be the primary and essential lesions in moyamoya disease. The etiology remains unknown. To detect possible mediators of the thickened intima of moyamoya disease, we measured serum alpha-1-antitrypsin (α1-AT) levels and characterized the phenotype of patients with familial moyamoya disease. *Patients and methods* Fifty-six individuals were examined, including 29 patients with moyamoya disease from 14 families. Serum α1-AT levels were analyzed by electroimmunoassay and genomic phenotype by isoelectric focusing. *Results:* All individuals had a normal α1-AT phenotype. The average serum α1-AT level in moyamoya disease patients was significantly higher than that of normal individuals, although both were within the normal range. *Conclusions:* These findings suggest that serum α1-AT level may be a marker, rather than an etiologic factor, indicating the progression of moyamoya disease.

Keywords Moyamoya disease · Alpha-1-antitrypsin

Introduction

The primary lesion in moyamoya disease is a progressive stenotic or occlusive change involving the circle of Willis and its major branches, including the distal segments of the internal carotid arteries [16]. The etiology of moyamoya disease remains unknown, although many clinical, pathological, pathophysiological, and genetic studies have been carried out.

Congenital factors have been considered to be important due to the high incidence among Asians and occasional familial occurrence. There is, however, no simple and useful marker to distinguish those who will develop moyamoya disease in familial cases, although several genetic factors seem to be significantly associated with moyamoya disease [2, 14, 17, 18, 32].

Intimal thickening due to smooth muscle cell proliferation and migration [16] is a primary structural abnormality in the major stenotic cerebral arteries in moyamoya disease. Moreover, similar lesions have been observed in vessels of the heart, kidney, and other organs [12] suggesting that systemic etiological factors are associated with moyamoya disease. Although the origin of moyamoya disease and the reason why it is limited to the major cerebral vessels remain unclear, several factors associated with the vascular wall, such as nitric oxide (NO), platelet-derived growth factor (PDGF) [1, 3], vascular endothelial growth factor (VEGF), or elastase [30] may be biochemically related to the etiology of moyamoya disease. Yamamoto et al. [29] have reported that elastin levels in smooth muscle cells are higher in patients with moyamoya disease. Elastin and collagen are the main components of the arterial wall, and an imbalance between a serum proteinase and its inhibitor may give rise to vascular matrix degeneration [6, 19]. Alpha-1-antitrypsin (α1-AT) is a serine protease inhibitor that primarily functions to protect tissue by inhibiting elastase or collagenase [21]. Quantitatively high levels of α1-AT can result in structural abnormalities of the vascular wall by excessive proliferation of smooth muscle cells and by syn-

thesis of vascular matrix components such as elastin and collagen. Low levels of α1-AT, on the other hand, can result in the destruction of elastin, collagen, and other connective tissue by increasing relative protease activity [21]. Ikari et al. [11] showed that α1-AT is a critical anti-apoptotic factor for vascular smooth muscle cells, while a deficiency of α1-AT has been reported to increase the risk of developing intracranial aneurysms [24, 25, 28] and fibromuscular dysplasia [26].

We hypothesized that serum levels and a genetic abnormality of α1-AT may affect the development of moyamoya disease.

Patients and methods

We studied 56 Japanese individuals (26 males and 30 females) from 14 families, each with 3–5 subjects. Each subject or the parents of the 14 families received informed consent and agreed to participate in this study. Twenty-nine (10 males and 19 females) had a definitive diagnosis of moyamoya disease (based on guidelines for the diagnosis of moyamoya disease established by the Research Committee on Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and Welfare of Japan) [15].

Serum α1-AT levels were determined by electroimmunoassay [20, 26]. α1-AT phenotypes were determined by isoelectric focusing in polyacrylamide gels at pH values between 3.5 and 5.0, using genomic DNA extracted from peripheral blood [20, 26].

Statistical analysis was performed using the Student's *t*-test. Differences with a p value <0.05 were considered statistically significant.

Results

The numbers of subjects with each α1-AT phenotype are listed in Table 1. The 'others' group in Table 1 consists of PiMM2, PiM1M1, PiM1M2, or PiGM alleles. All individuals had the normal M phenotypes except for one with a rare normal PiGM allele. None of the subjects had abnormal phenotypes such as PiZ or PiS, causing quantitative deficiency of α1-AT levels. Thus, no specific α1-AT phenotype appears to be associated with moyamoya disease.

The average serum α1-AT levels of normal individuals and of individuals with moyamoya disease were 131.1±23.6 (mean ± SD) and 152.0±39.1 mg/dl respectively (Table 1). Patients with moyamoya disease showed significantly higher values than normal individuals (p<0.05). The average serum α1-AT levels in both groups, however, were within the normal range (from 100 to 190 mg/dl). Only 4 individuals had values above 190 mg/dl (3 moyamoya patients and 1 normal individual, data not shown). None of the subjects had decreased levels of serum α1-AT (below 100 mg/dl).

Discussion

Moyamoya disease occurs predominantly in people of Asian origin and the incidence of familial occurrence has been estimated to be 10% [9, 17, 34]. A massive case control study by the Research Committee on Moyamoya Disease in Japan failed to show any positive associations with environmental agents, including previous infections [10, 13, 33]. Therefore, certain genetic factors are likely to be related to the etiology of moyamoya disease, but have thus far remained unknown.

We investigated serum α1-AT levels and phenotypes in familial moyamoya disease. More than 75 different α1-AT alleles are known, which qualitatively and quantitatively affect α1-AT protein [8, 21]. Most normal individuals have the M phenotype (M, M1, or M2), expressing normal levels of α1-AT. The most common alleles associated with a quantitative deficiency of α1-AT are PiZ and PiS, resulting in pulmonary emphysema or liver cirrhosis [5, 7, 26]. In contrast, particular alleles associated with an excess of α1-AT have not been identified. A deficiency of serum α1-AT associated with genetic factors may cause degradation of the arterial wall, through inadequate protection against protease activity [5, 22, 26]. In the present study, however, all individuals had the normal α1-AT phenotype including a rare normal GM phenotype, indicating that there was no specific genetic abnormality of α1-AT in association with familial moyamoya disease. Thus, it is not possible to use α1-AT phenotype to predict those susceptible family members who may develop moyamoya disease.

On the other hand, the average serum α1-AT level of moyamoya disease patients was comparatively higher, although it was not above the normal range. α1-AT is also recognized as an acute phase reactant similar to C reactive protein (CRP), increasing in response to the

Table 1 The number of alpha-1-antitrypsin phenotype and the average serum alpha-1-antitrypsin level (*α1-AT* alpha-1-antitrypsin, *ns* not significant)

α1-AT phenotype	Moyamoya disease		Normal individuals	
	n	Serum α1-AT level (mg/dl)[a]	*n*	Serum α1-AT level (mg/dl)[a]
M	7	112.9±6.2 (ns)	5	108.8±7.5
MM	11	155.5±35.9*	7	131.9±11.5
MM1	8	151.4±47.7*	11	131.0±29.4
Others	3	136.0±11.8	4	147.5±47.9
Total	29	152.0±39.1**	27	131.1±23.6

[a] Mean±SD
*p<0.01; **p<0.05

general stimulus of inflammation [4]. Previous reports have suggested that inflammatory stimuli and the subsequent response of inflammatory cells may produce a proliferative response in smooth muscle cells, leading to the thickened intima in patients with moyamoya disease [23, 27, 31]. In our study, serum samples were obtained from moyamoya disease subjects during the development of ischemic changes. The quantitative changes in serum α1-AT might reflect tissue damage or inflammation in the progressive state of moyamoya disease, rather than being a primary cause of moyamoya disease. However, continuously high levels of α1-AT may also result in structural abnormalities of the vascular wall due to excessive proliferation of smooth muscle cells and synthesis of vascular matrix components such as elastin and collagen. Thus, further study is necessary to clarify:

1. The relationship between serum α1-AT level and severity of ischemia in moyamoya disease
2. The chronological changes in serum α1-AT levels
3. The involvement of other serine protease inhibitors

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