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A follow-up study on relationship between  
respiratory symptoms and serum concentrations of  
protease inhibitors in Japanese children

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SUMMARY

In the US and European countries, serum protease inhibitors deficiency has been recognized as a risk factor for chronic obstructive pulmonary disease. To clarify the fundamental relationship between some serum proteins and respiratory symptoms in Japanese children, a four-year follow-up study has been conducted. The subjects were first to third grade school children in 1992 attending three elementary schools in Kimitsu, Chiba Prefecture, Japan. Their respiratory symptoms were assessed from responses to annual questionnaires. The serum  $\alpha_1$ -antitrypsin ( $\alpha_1$ AT),  $\alpha_2$ -macroglobulin ( $\alpha_2$ MG), and albumin concentrations were determined. This study has analyzed 652 children (327 boys, 325 girls) whose questionnaires and blood results were successfully completed. A decreased level of serum  $\alpha_1$ AT in 1992 was significantly associated with the prevalence of asthma and wheezing and a history of allergic diseases as of 1995. No relationship of serum  $\alpha_2$ MG levels with respiratory symptoms or allergic diseases was observed. The mean levels of serum albumin were significantly lower in girls with asthma than in those without. These findings suggest that serum  $\alpha_1$ AT and albumin levels are associated with respiratory and allergic diseases in children. A decreased  $\alpha_1$ AT level should be considered as a biological risk marker for these diseases.

**Key words:** asthma, allergic diseases,  $\alpha_1$ -antitrypsin,  $\alpha_2$ -macroglobulin, albumin

**Abbreviations:**  $\alpha_1$ AT:  $\alpha_1$ -antitrypsin

$\alpha_2$ MG:  $\alpha_2$ -macroglobulin

COPD: chronic obstructive pulmonary disease

ETS: environmental tobacco smoke

ANOVA: analysis of variance

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## I. Introduction

Both  $\alpha_1$ -antitrypsin ( $\alpha_1$ AT) and  $\alpha_2$ -macroglobulin ( $\alpha_2$ MG) are major serum inhibitors of proteases including trypsin, plasmin, and elastase[1-4]. They are known to play important roles in the defense of the host by protecting the tissues from potential enzymatic damage. In the US and European countries,  $\alpha_1$ AT deficiency has been recognized as an important risk factor for chronic obstructive pulmonary disease (COPD) [5,6]. The relationship between a serum  $\alpha_1$ AT level and bronchial asthma has also been described[7].

The distribution of serum protease inhibitors is known to vary between races[8]. In Japanese people  $\alpha_1$ AT deficiencies are very rare. Only several cases of  $\alpha_1$ AT deficiency have been reported in Japan[9]. Almost all Japanese people have identical  $\alpha_1$ AT phenotype pattern[10]. Few researchers have so far measured the serum  $\alpha_1$ AT concentrations among Japanese children. Very little information is available regarding the relationship between serum protease inhibitors levels and respiratory symptoms in Japanese children.

Shima and Adachi[11] reported that serum concentrations of  $\alpha_1$ AT,  $\alpha_2$ MG, and albumin were associated with respiratory and allergic diseases among schoolchildren living in Chiba

Prefecture, Japan. A decreased  $\alpha_1$ AT level should be considered as a biological risk marker for these diseases. However, it was pointed out that further examination should be made in a follow-up study to clarify these findings.

The purposes of the present study were to monitor and examine further the schoolchildren living in Chiba Prefecture, Japan, who were examined in the previous study[11]. Special emphases were placed on ; (1) confirming if serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin levels of the children vary according to age, (2) identifying the children with  $\alpha_1$ AT deficiency, (3) clarifying if a decreased or increased  $\alpha_1$ AT level should be considered as a possible risk factor for respiratory and allergic diseases, and (4) investigating the relationship between serum protease inhibitors levels and the intensity of exposure to environmental tobacco smoke (ETS).

## II. Materials and Methods

### Subjects (Table 1)

In the year of 1992, the examination of the first to third grade children attending three elementary schools (aged 6-9 years) in Kimitsu, Chiba Prefecture, Japan, was implemented. The concentrations of these air pollutants were below the Japanese Ambient Air Quality Standard from 1992 to 1993, during the time this

Table 1 The number of subjects according to sex, school grade and year.

Year	1992	1994	1995
Boys	93 (1st grade) 71 (2nd grade) 87 (3rd grade)	114 (3rd grade) 77 (4th grade) 93 (5th grade)	132 (4th grade) 88 (5th grade) 107 (6th grade)
Subtotal(n)	251	284	327
Girls	78 (1st grade) 72 (2nd grade) 89 (3rd grade)	86 (3rd grade) 79 (4th grade) 96 (5th grade)	109 (4th grade) 102 (5th grade) 114 (6th grade)
Subtotal(n)	239	261	325
Total(n)	490	545	652

study was conducted. In order to follow up these sample groups, children attending the same elementary schools were examined in the year of 1994 and 1995. Therefore, the third to fifth grade children and the fourth and sixth grade children in the same schools were investigated in 1994 and 1995, respectively.

#### *Data Collection and Laboratory Measurements*

In November 1992, October 1994 and October 1995, standard respiratory symptom questionnaires, the modified Japanese version of ATS-DLD-78-C, were sent out to all the subjects. Either their parents or guardians filled them out. The questionnaires covered respiratory symptoms and medical histories of the children, and the smoking habits of household members. If any members of the household were smokers, the respondent was asked to note the number of cigarettes that each of them smoked per day in the present of the child.

According to the responses to the questionnaire, children who had experienced two or more episodes of wheezing accompanied by dyspnea, or had been receiving medical treatment for bronchial asthma during the past two years, were considered to have asthma. Children with no history of asthma who reported any wheezing symptoms during the past two years were considered to have wheezing symptoms. Those who had been diagnosed as having eczema,

atopy, allergic rhinitis, or pollinosis by a physician, or had received anti-allergic therapy, such as hyposensitization, were considered to have a history of allergic diseases. As for the intensity of exposure to ETS, the subjects were categorized into three groups; children in households in which there were no smokers, those in which there were smokers but the total number of cigarettes smoked in the presence of the child was  $\leq 10$  a day, and those in which the total number of cigarettes smoked in the presence of the child was  $\geq 11$  a day.

In January 1993, December 1994 and November 1995, venous blood samples were collected from the children whose parents or guardians had given their written consent. The blood specimens were centrifuged on the same day, and all serum samples were kept frozen at  $-80^{\circ}\text{C}$  until analyzed. The concentrations of serum  $\alpha_1\text{AT}$ ,  $\alpha_2\text{MG}$  and albumin were determined by a nephelometric method using a Behring Nephelometer Analyzer (Dade Behring, Marburg, Germany). In 1994, the concentrations of serum albumin were not been determined.

As shown in Fig.1, in 1995 the questionnaires of 652 children were completed and returned. Blood samples were collected from them all but seven children from whom  $\alpha_2\text{MG}$  tests blood samples were not drawn. In 1994, out of the 652 children in 1995, the blood test data were collected from 545 children. The blood samples

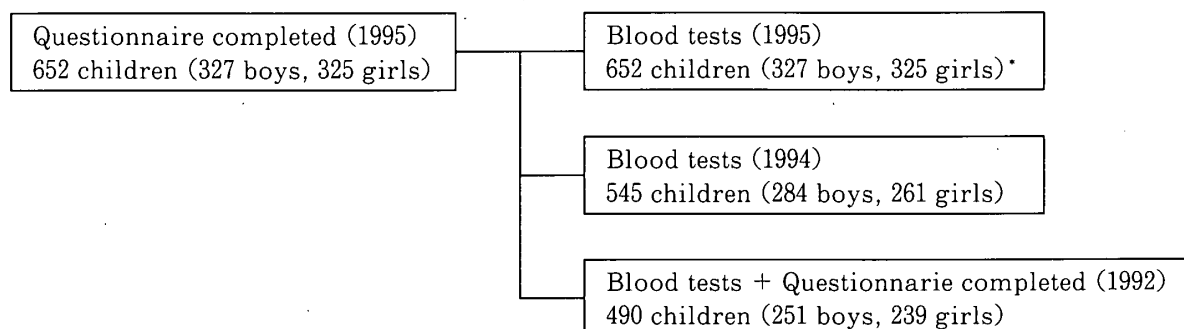


Fig. 1 Composition of the subjects.

\* Serum  $\alpha_2\text{MG}$  of seven children were not examined.

were collected from 490 children in 1992 out of the above-mentioned 652 children.

### Data Analysis

Firstly, the levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin were evaluated with analysis of variance (ANOVA) and linear regression analysis in relation to sex, school grade and year (1992, 1994 and 1995). If any significant findings were observed among groups, Tukey-Kramer test was conducted for testing the differences between the groups.

Then, an attempt was made to identify the number of children with  $\alpha_1$ AT deficiency among the subjects. In order to determine if a decreased or increased  $\alpha_1$ AT level can be a risk marker for the respiratory diseases, the relationships of the number of children with serum  $\alpha_1$ AT levels of  $<160\text{mg/dl}$  or  $\geq 240\text{mg/dl}$  to the prevalence of respiratory symptoms and allergic histories were investigated. In this case, significance of the trend was evaluated by Cochran-Armitage method. Furthermore, the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin were analyzed in relation to the respiratory symptoms (asthma or wheezing) and allergic histories. The  $t$  distribution was also applied to compare the serum mean levels in the children with histories of allergic diseases to those in the children without.

Finally, evaluation was made on the association of the levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG, and albumin with the ETS. Statistical analyses were conducted using JMP programs (SAS Institute, Cary, NC).

### III. Results

Table 2 indicates the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to school grade. In boys of the year of 1992, the mean level of serum  $\alpha_1$ AT in the 4th grade was significantly higher than that in the 6th grade. In

both boys and girls in the year of 1995, the mean levels were found to be significantly higher in the 4th grade than those in the other grades. Linear regression analysis revealed that the mean levels have significantly declined with increasing grade in boys of the year 1992 and both sexes of the year 1995. On the contrary, no significant relationship between the mean levels and the grades was observed in girls of the year 1992 or both sexes of the year 1994.

In 1992, the mean levels of serum  $\alpha_2$ MG of boys and girls in the 4th grade were significantly higher than those in the 6th grade and the other grades, respectively. In boys of the year of 1995, the mean level of serum  $\alpha_2$ MG was found to be significantly lower in the 4th grade than that in the 5th grade. Linear regression analysis showed the mean levels in boys and girls of the year 1992 have significantly declined with increasing grade.

In both boys and girls in the year of 1995, the mean levels of serum albumin in the 4th grade were found to be significantly higher than those in the other grades. According to linear regression analysis, it indicated that the mean levels have significantly declined with increasing grade.

Table 3 shows the prevalence of asthma and wheezing symptoms, and the number of children with histories of allergic diseases in relation to serum  $\alpha_1$ AT levels. This purpose is to determine whether a decreased ( $<160\text{mg/dl}$ ) or increased ( $\geq 240\text{mg/dl}$ ) serum  $\alpha_1$ AT level can become a predictor for the prevalence of asthma and wheezing symptoms, and allergic diseases.

As a result, no children with  $\alpha_1$ AT deficiency have been detected in 1992, 1994 or 1995. In 1992, out of 490 children, 12 children (2.4%) with serum  $\alpha_1$ AT levels of  $<160\text{mg/dl}$  were detected. In 1994 and 1995, 3 (0.6%) out of 545 children and 15 (2.3%) out of 652 children with serum  $\alpha_1$ AT levels of  $<160\text{mg/dl}$  were found, respectively. Each time the examination was

Table 2 The mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to school grade.

Year	1992			1994			1995		
	n	$\bar{x}$	(s)	n	$\bar{x}$	(s)	n	$\bar{x}$	(s)
<b>Boys</b>									
$\alpha_1$ AT(mg/dl)									
4th grade	93	216.0	(34.2)	114	232.4	(37.0)	132	231.1	(59.6)
5th grade	71	211.1	(26.4)	77	239.0	(35.9)	88	207.5	(27.7)
6th grade	87	204.5	(26.4)	93	239.4	(33.7)	107	208.4	(32.7)
P-value*		0.010			0.151			<0.001	
$\alpha_2$ MG(mg/dl)									
4th grade	93	382.5	(55.9)	114	404.9	(53.3)	132	328.9	(54.8)
5th grade	71	373.3	(47.5)	77	420.4	(47.6)	88	357.9	(49.1)
6th grade	87	356.7	(42.9)	93	417.1	(63.5)	107	343.5	(57.5)
P-value*		<0.001			0.101			0.028	
Albumin(mg/dl)									
4th grade	93	4893.1	(355.1)				132	5588.4	(840.1)
5th grade	71	4971.3	(303.7)				88	5172.6	(746.6)
6th grade	87	4969.2	(287.4)				107	5179.5	(853.1)
P-value*		0.107						<0.001	
<b>Girls</b>									
$\alpha_1$ AT(mg/dl)									
4th grade	78	213.3	(38.3)	86	230.8	(36.2)	109	244.9	(57.5)
5th grade	72	209.9	(32.8)	79	241.1	(34.4)	102	215.6	(31.9)
6th grade	89	206.7	(30.3)	96	236.9	(36.6)	114	207.4	(32.6)
P-value*		0.147			0.164			<0.001	
$\alpha_2$ MG(mg/dl)									
4th grade	78	376.3	(65.7)	86	377.3	(46.8)	102	314.6	(61.4)
5th grade	72	354.9	(37.6)	79	386.4	(49.7)	102	322.9	(40.3)
6th grade	89	338.6	(44.5)	96	381.9	(65.7)	114	215.2	(48.7)
P-value*		<0.001			0.432			0.963	
Albumin(mg/dl)									
4th grade	78	4968.7	(330.9)				109	6030.2	(979.0)
5th grade	72	5048.3	(273.6)				102	5148.9	(759.2)
6th grade	89	5025.5	(335.5)				114	5173.2	(853.8)
P-value*		0.262						<0.001	

\* Statistical analysis by ANOVA followed by Tukey-Kramer method

#1  $P < 0.05$  compared with that in the 5th grade#2  $P < 0.05$  compared with that in the 6th grade.  $P < 0.05$  Significance of the trend was evaluated by linear regression analysis

conducted, only one child showed the constant  $<160$ mg/dl of serum  $\alpha_1$ AT level. This child had no respiratory symptoms as of both 1992 and 1995 but had a history of allergic disease.

As for the number of children with serum  $\alpha_1$ AT levels of  $\geq 240$ mg/dl in 1992, 73 children (14.9%) out of 490 children were detected. In 1994 and 1995, out of 545 and 652 children, 219

(40.1%) and 151 children (23.1%) with serum  $\alpha_1$ AT levels of  $\geq 240$ mg/dl were found, respectively. Each time the examination was conducted in the year of 1992, 1994 and 1995, nine children with  $\geq 240$ mg/dl of serum  $\alpha_1$ AT levels were found. Seven children out of them had no respiratory symptoms as of both 1992 and 1995. Two children of them had asthma as of either

Table 3 Prevalence of asthma and wheezing in relation to the levels of  $\alpha_1$ AT.

Symptoms	$\alpha_1$ AT<160mg/dl	160mg/dl $\leq\alpha_1$ AT<240mg/dl	$\alpha_1$ AT $\geq$ 240mg/dl	total	P-value*
1992, <i>n</i>	12	405	73	490	
As of 1992					
Asthma	3 ( 25.0%)	23 ( 5.7%)	9 ( 12.3%)	35 ( 7.1%)	0.468
Wheezing	0 ( 0.0%)	12 ( 3.0%)	2 ( 2.7%)	14 ( 2.9%)	0.861
Allergic histories	12 (100.0%)	220 ( 54.3%)	34 ( 46.6%)	266 ( 54.3%)	0.011
As of 1995					
Asthma	4 ( 33.3%)	29 ( 7.2%)	3 ( 4.1%)	36 ( 7.3%)	0.017
Wheezing	3 ( 25.0%)	18 ( 4.4%)	1 ( 1.4%)	22 ( 4.5%)	0.010
Allergic histories	9 ( 75.0%)	231 ( 57.0%)	29 ( 39.7%)	269 ( 54.9%)	0.002
1994, <i>n</i>	3	323	219	545	
As of 1992 <sup>#1</sup>					
Asthma	0 ( 0.0%)	18 ( 6.9%)	13 ( 7.6%)	31 ( 7.2%)	0.718
Wheezing	0 ( 0.0%)	11 ( 4.2%)	2 ( 1.2%)	13 ( 3.0%)	0.087
Allergic histories	1 ( 33.3%)	146 ( 56.4%)	86 ( 50.3%)	233 ( 53.8%)	0.299
As of 1995					
Asthma	0 ( 0.0%)	25 ( 7.7%)	15 ( 6.8%)	40 ( 7.3%)	0.779
Wheezing	0 ( 0.0%)	18 ( 5.6%)	10 ( 4.6%)	28 ( 5.1%)	0.670
Allergic histories	2 ( 66.7%)	172 ( 53.3%)	131 ( 59.8%)	305 ( 56.0%)	0.101
1995, <i>n</i>	15	486	151	652	
As of 1992 <sup>#2</sup>					
Asthma	0 ( 0.0%)	26 ( 7.1%)	9 ( 7.9%)	35 ( 7.1%)	0.530
Wheezing	0 ( 0.0%)	12 ( 3.3%)	2 ( 1.8%)	14 ( 2.9%)	0.578
Allergic histories	7 ( 63.6%)	204 ( 55.9%)	55 ( 48.2%)	266 ( 54.3%)	0.118
As of 1995					
Asthma	1 ( 6.7%)	41 ( 8.4%)	6 ( 4.0%)	48 ( 7.4%)	0.102
Wheezing	2 ( 13.3%)	21 ( 4.3%)	10 ( 6.6%)	33 ( 5.1%)	0.664
Allergic histories	10 ( 66.7%)	279 ( 57.4%)	71 ( 47.0%)	360 ( 55.2%)	0.016

\* $P<0.05$  Significance of the trend was evaluated by Cochran-Armitage method

<sup>#1</sup> The number of children in 1994 whose data were not obtained as of 1992 was 64 and 48 in the categories of 160mg/dl $\leq\alpha_1$ AT<240mg/dl and  $\alpha_1$ AT $\geq$ 240mg/dl, respectively.

<sup>#2</sup> The number of children in 1995 whose data were not obtained as of 1992 was 4, 121 and 37 in the categories of  $\alpha_1$ AT<160mg/dl, 160mg/dl $\leq\alpha_1$ AT<240mg/dl and  $\alpha_1$ AT $\geq$ 240mg/dl, respectively.

1992 or 1995. The percentage of the children in 1992 with a history of allergic diseases has significantly declined with the increasing levels of serum  $\alpha_1$ AT as of 1992 and 1995. As of 1995, the prevalence of asthma and wheezing symptoms has significantly declined with the increasing levels of serum  $\alpha_1$ AT. In 1994 and 1995, significant differences were not observed.

Table 4 indicates the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to the respiratory symptoms (asthma and wheezing), which have been obtained in 1995 surveys. The purpose of this table is to clarify the

association of the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin with the presence of respiratory symptoms.

In 1992 the mean level of serum  $\alpha_1$ AT in girls with the prevalence of asthma was exclusively and significantly lower than in those without. No significant findings were detected in the relationship between the mean level of serum  $\alpha_1$ AT and the prevalence of wheezing. As for the relationships of mean levels of serum  $\alpha_2$ MG with the children with the prevalence of asthma and wheezing, any significant findings were not observed. The mean level of serum

Table 4 The mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in 1995 in relation to asthma or wheezing.

Year	1992			1994			1995		
	<i>n</i>	$\bar{x}$	(s)	<i>n</i>	$\bar{x}$	(s)	<i>n</i>	$\bar{x}$	(s)
Boys									
$\alpha_1$ AT(mg/dl)									
Asthma	20	197.9	( 30.0)	26	226.5	( 32.3)	30	214.4	( 42.4)
Wheezing	13	201.4	( 30.4)	15	245.3	( 43.8)	19	229.1	( 53.4)
No symptom	218	212.4	( 29.5)	243	237.0	( 35.5)	278	216.8	( 45.8)
$\alpha_2$ MG(mg/dl)									
Asthma	20	387.2	( 56.9)	26	406.1	( 55.6)	30	332.8	( 52.9)
Wheezing	13	346.8	( 27.0)	15	421.7	( 75.5)	19	340.0	( 77.3)
No symptom	218	370.9	( 50.4)	243	413.3	( 54.5)	278	342.5	( 54.0)
Albumin(mg/dl)									
Asthma	20	4780.0	(276.6)				30	5091.0	( 540.9)
Wheezing	13	4966.2	(296.7)				19	5589.5	(1234.1)
No symptom	218	4955.0	(321.5)				278	5353.0	( 833.6)
Girls									
$\alpha_1$ AT(mg/dl)									
Asthma	16	182.8	( 40.2)*	14	234.9	( 37.6)	18	206.6	( 34.5)
Wheezing	9	186.5	( 48.0)	13	231.8	( 35.2)	14	215.5	( 52.6)
No symptom	214	212.8	( 31.5)	234	236.5	( 36.0)	293	223.9	( 45.5)
$\alpha_2$ MG(mg/dl)									
Asthma	16	359.1	( 73.3)	14	387.6	( 43.5)	18	311.7	( 37.2)
Wheezing	9	379.3	( 84.6)	13	379.2	( 57.6)	14	314.1	( 63.9)
No symptom	214	354.6	( 49.5)	234	381.6	( 55.9)	286	318.0	( 50.9)
Albumin(mg/dl)									
Asthma	16	4763.1	(295.7)*				18	5164.4	( 938.3)
Wheezing	9	5036.7	(371.2)				14	5405.7	(1349.7)
No symptom	214	5031.6	(309.6)				293	5473.0	( 940.9)

\*  $P < 0.05$  Statistical analysis by ANOVA followed by Tukey-Kramer method

albumin was significantly lower in girls of 1992 with the prevalence of asthma than in girls without. With regard to both boys and girls with the prevalence of wheezing in the year of 1992 and 1995, no significant findings were detected.

Table 5 indicates the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to the histories of allergic diseases, which have been conducted in 1995 surveys. This shows the association of the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin with the presence of allergic histories. The mean levels of serum  $\alpha_1$ AT were exclusively and significantly lower in boys with

a history of allergic diseases in 1992 than in boys without. As for the relationships of mean levels of serum  $\alpha_2$ MG with the children with allergic histories, any significant findings were not detected. Concerning allergic histories in the year of 1992 and 1995, no significant findings were detected.

Table 6 indicates the mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to the intensity of exposure to ETS, which has been obtained in 1995 surveys. No significant findings were detected except in girls of the year 1992 in which the level of serum  $\alpha_1$ AT in the group of no exposure was significantly higher

Table 5 The mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in 1995 in relation to a history of allergic diseases

Year	1992			1994			1995		
	<i>n</i>	$\bar{x}$	(s)	<i>n</i>	$\bar{x}$	(s)	<i>n</i>	$\bar{x}$	(s)
Boys									
$\alpha_1$ AT(mg/dl)									
No	111	216.1	( 30.2)	111	233.5	( 36.4)	137	218.8	( 41.4)
Yes	140	206.4	( 28.9)	173	238.4	( 35.2)	190	216.3	( 49.0)
<i>P</i> -value*		0.010			0.258			0.622	
$\alpha_2$ MG(mg/dl)									
No	111	375.0	( 46.6)	111	416.3	( 58.2)	137	346.5	( 52.5)
Yes	140	367.8	( 53.2)	173	411.0	( 54.1)	190	337.8	( 57.2)
<i>P</i> -value*		0.264			0.432			0.160	
Albumin(mg/dl)									
No	111	4956.9	(335.9)				137	5377.7	(812.2)
Yes	140	4929.4	(306.4)				190	5317.5	(865.1)
<i>P</i> -value*		0.061						0.525	
Girls									
$\alpha_1$ AT(mg/dl)									
No	110	212.4	( 34.7)	129	236.2	( 33.5)	155	226.2	( 48.3)
Yes	129	207.6	( 33.0)	132	236.2	( 38.2)	170	219.3	( 42.3)
<i>P</i> -value*		0.268			0.992			0.172	
$\alpha_2$ MG(mg/dl)									
No	110	357.8	( 54.7)	129	383.0	( 57.3)	151	317.9	( 47.1)
Yes	129	354.1	( 51.4)	132	380.6	( 53.3)	167	317.1	( 54.0)
<i>P</i> -value*		0.599			0.726			0.888	
Albumin(mg/dl)									
No	110	5030.5	(309.2)				155	5408.3	(958.3)
Yes	129	4999.7	(323.9)				170	5493.8	(963.2)
<i>P</i> -value*		0.456						0.424	

\*  $P < 0.05$  The *t*-distribution was used to test the relationship of the children with a history of allergic diseases to those without.

than that in the category for low exposure.

In boys of the year in 1994, the levels differed significantly among the groups of serum  $\alpha_2$ MG. But no significant differences between the groups were indicated by Tukey-Kramer test. According to linear regression analysis, in boys of the year 1994, the serum  $\alpha_2$ MG levels were found to have significantly decreased with the increase of intensity of exposure to ETS. The levels of serum albumin in boys of the year 1992 and in girls of the year 1995 tended to decline with the increase of the intensity of ETS, but these changes were not significant.

#### IV. Discussion

Shima and Adachi[11] suggested that some individuals are more susceptible than others to the effects of environmental factors, although the physiological reasons for this are unknown. In their study, in order to clarify the susceptibility the fundamental association between some serum proteins and respiratory symptoms in Japanese children was investigated. It was suggested that serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin levels are associated with respiratory symptoms and allergic diseases in children. A



Table 6 The mean levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin in relation to the intensity of ETS.

Year	1992			1994		1995		
	$\alpha_1$ AT (mg/dl)	$\alpha_2$ MG (mg/dl)	Albumin (mg/dl)	$\alpha_1$ AT (mg/dl)	$\alpha_2$ MG (mg/dl)	$\alpha_1$ AT (mg/dl)	$\alpha_2$ MG (mg/dl)	Albumin (mg/dl)
Boys								
null								
<i>n</i>	102	102	102	117	117	136	136	136
$\chi$	210.6	377.4	4944.5	233.2	423.3	217.0	343.9	5252.0
(s)	28.3	50.0	322.2	32.5	53.2	46.4	55.1	781.1
1-10								
<i>n</i>	123	123	123	140	140	157	157	157
$\chi$	210.5	365.2	4941.6	240.3	407.5	218.2	339.2	5397.0
(s)	28.6	47.8	312.4	38.6	53.8	47.3	55.7	858.6
11 $\leq$								
<i>n</i>	26	26	26	27	27	34	34	34
$\chi$	211.5	372.9	4930.0	231.2	397.4	214.7	342.3	5455.0
(s)	40.8	61.5	352.5	32.5	69.2	38.1	56.5	986.1
<i>P</i> -value <sup>#3</sup>	0.925	0.242	0.855	0.524	0.007 <sup>#1</sup>	0.936	0.651	0.103
Girls								
null								
<i>n</i>	93	93	93	96	96	130	127	130
$\chi$	216.6	360.3	5021.0	235.0	373.2	226.6	320.7	5561.5
(s)	32.6	56.3	320.0	33.7	54.2	47.0	56.7	1075.0
1-10								
<i>n</i>	132	132	132	150	150	173	170	173
$\chi$	204.3	353.6	5006.0	236.2	386.5	218.7	315.5	5389.0
(s)	35.0	51.6	317.4	37.4	54.8	44.3	47.4	899.4
11 $\leq$								
<i>n</i>	14	14	14	15	15	22	21	22
$\chi$	216.4	347.5	5040.7	243.5	388.8	229.0	313.0	5315.5
(s)	19.9	40.9	311.6	35.6	63.0	42.3	39.2	614.0
<i>P</i> -value <sup>#3</sup>	0.075	0.260	0.920	0.497	0.070	0.431	0.343	0.098

<sup>#1,2</sup>  $P < 0.05$  Statistical analysis by ANOVA followed by Tukey-Kramer method.

<sup>#1</sup> Significant difference was not observed by Tukey-Kramer method.

<sup>#2</sup>  $P < 0.05$  Significant difference was observed between (null) and (1-10).

<sup>#3</sup>  $P < 0.05$  Significance of the trend was evaluated by linear regression analysis.

decreased  $\alpha_1$ AT should be considered as a biological risk marker for these diseases. Furthermore, to determine the relationships of these biochemical markers to the etiology of diseases, the involvement of both genetic and environmental factors should be further evaluated in cohort studies.

To follow up the study [11], we have monitored and examined the same sample group in 1994 and 1995. In other countries, some studies have continued for long years and covered a large population size. Piitulainen and Sveger

[12] have reported that individuals identified in the Swedish neonatal  $\alpha_1$ AT screening study were followed prospectively from their first to their eighteenth year of life. Such a large number of Japanese children have not yet been followed previously.

Wagnerova et al [13] showed that serum  $\alpha_1$ AT levels in children increased with age, even though their levels had been markedly lower at age 11-13 years. Shima and Adachi [11] have reported that the concentrations of serum protease inhibitors and albumin differed

according to sex and grade. They have adjusted the mean levels by grade in their study. As for the relationship between the levels of serum  $\alpha_2$ MG and age, it is thought to participate in the growth of children although the physiological role of human  $\alpha_2$ MG has not been elucidated [3].

The significant findings in the present study suggested that the levels of serum  $\alpha_1$ AT and  $\alpha_2$ MG might be shifted with the age, although the mean levels of serum  $\alpha_1$ AT and  $\alpha_2$ MG by grade have not been adjusted. To clarify the relationship of the levels of serum protease inhibitors and albumin to school grade, further examination should be further conducted.

Shima and Adachi[11] reported that they found no child with a  $\alpha_1$ AT deficiency. In the present study no child with a  $\alpha_1$ AT deficiency has been detected. These findings supported that the children with  $\alpha_1$ AT deficiency among Japanese are very rare[9]. However, they reported that fourteen children (2.9%) out of 480 children were found to have decreased serum  $\alpha_1$ AT concentrations of  $<160$ mg/dl, and all of them had histories of allergic diseases[11]. Serum  $\alpha_1$ AT levels were significantly related to the prevalence of asthma and histories of allergic diseases. In addition, it was revealed that 54 children (11.3%) out of 480 children were found to have increased serum  $\alpha_1$ AT concentrations of  $\geq 240$ mg/dl. The percentage of children with a history of allergic diseases became significantly lower with increasing  $\alpha_1$ AT levels.

In this study, in 1994 and 1995, 3 (0.6%) children and 15 (2.3%) with serum  $\alpha_1$ AT levels of  $<160$ mg/dl were found, respectively. In 1995, 151 children (23.1%) with serum  $\alpha_1$ AT levels of  $\geq 240$ mg/dl were found. The percentage of the children in 1992 with a history of allergic diseases has significantly declined with the increasing levels of serum  $\alpha_1$ AT as of 1992 and 1995. As of 1995, the prevalence of asthma and wheezing symptoms has significantly declined

with the increasing levels of serum  $\alpha_1$ AT. In 1994 and 1995, significant differences were not observed in the prevalence of asthma and wheezing and the percentage of the children in relation to a history of allergic diseases.

The mean serum  $\alpha_1$ AT levels were significantly lower in girls with asthma, and in both boys and girls who had histories of allergic diseases. However, no association was found between mean serum  $\alpha_1$ AT levels and the prevalence of wheezing. In 1992 the mean level of serum  $\alpha_1$ AT in girls with the prevalence of asthma was exclusively and significantly lower than in those without. The mean levels of serum  $\alpha_1$ AT in boys with a history of allergic diseases in 1992 were exclusively and significantly lower than in the children without.

Considering the distribution of serum  $\alpha_1$ AT levels and the mean levels of serum  $\alpha_1$ AT in relation to the presence of asthma, wheezing and allergic histories as was already mentioned above, it was suggested that the declined level of serum  $\alpha_1$ AT in the children should be possible risk factors for asthma and allergic diseases in Japanese children. However, we should be careful because the results in 1992 were partly overlapped with those observed in the previous study[11]. In addition, the level of 160 mg/dl was used as a cut-off point for a decreased  $\alpha_1$ AT level. As a result, its sensitivity might be considered pretty low. Therefore, an appropriate cut-off point for a decreased  $\alpha_1$ AT level should be further examined.

In the previous study[11], there was no difference in the grade-adjusted mean levels of serum  $\alpha_2$ MG in relation to the presence of asthma or wheezing symptoms. However, serum  $\alpha_2$ MG levels in children with a history of allergic diseases were higher than in those without, and this difference was significant in girls. The physiological role of human  $\alpha_2$ MG has not been elucidated. Umeki et al[14] found no change in serum  $\alpha_2$ MG levels in-patients

with bronchial asthma. In contrast, it was indicated that increased concentrations of  $\alpha_2$  MG might, to some extent, compensate for the protease/protease inhibitor imbalance in the  $\alpha_1$ AT deficiency state[15,16]. In the present study, as for the relationships of mean levels of serum  $\alpha_2$ MG with the children with the prevalence of asthma and wheezing and allergic histories, no significant findings were detected. Therefore, the relationship between serum  $\alpha_2$  MG levels and allergic diseases should be evaluated further.

Albumin is considered as a negative acute phase reactant since its level decreases in response to inflammation in the body[17,18]. In the previous study[11], serum albumin levels declined significantly in children with asthma, although they did not differ in relation to histories of allergic diseases. In this study, as for the children with the prevalence of asthma, only in girls of 1992, the mean level of serum albumin was significantly lower than in the children without. With regard to both boys and girls with the prevalence of wheezing and allergic histories in the year of 1992 and 1995, no significant findings were detected. It was suggested that a decreased albumin level observed in the children with asthma should reflect an inflammatory reaction in their bodies.

Many studies have investigated changes in protease inhibitors levels in the serum or the BAL fluid of smokers[19-21]. Most of these studies showed that serum  $\alpha_1$ AT levels were elevated in smokers. Shima and Adachi[11] concluded that serum  $\alpha_1$ AT levels were slightly increased in children exposed to ETS from  $>11$  cigarettes a day, but they did not significantly differ in relation to the intensity of exposure to ETS.

In this follow-up study, no relationship of the levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin to the intensity of exposure to ETS were observed in 1992, 1994 or 1995. This may be

because the effect of exposure to ETS is small compared with that of actual smoking. In addition, it was reported that tobacco smoking is the major risk factor for developing COPD, which generally begins by the third decade of life, much earlier than usual COPD that occurs in  $\alpha_1$ AT-replete individuals[22]. Therefore, it can be too early to determine the effect of ETS to the children in relation to the levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and albumin. A long-term follow-up study up to adults needs to be conducted.

Piitulainen and Sveger[12] reported that, after analyzing the effect of environmental factors on the occurrence of respiratory symptoms in  $\alpha_1$ AT-deficient adolescents, parental smoking might contribute to decreased lung function. Many have so far reported that the passive exposure to parental or maternal cigarette smoke might have important effects on the development of pulmonary function in children[23-30]. Further investigation is needed to clarify the involvement of serum protease inhibitors with the effects of the passive smoking on the pulmonary function.

## V. Conclusions

- (1) A four-year follow-up study has been conducted to clarify the relationship between serum proteins and respiratory symptoms in Japanese children.
- (2) No children with a  $\alpha_1$ AT deficiency have been detected among the subjects in this study.
- (3) A decreased level of serum  $\alpha_1$ AT was associated with the prevalence of asthma and a history of allergic diseases.
- (4) No relationship between serum  $\alpha_2$  MG levels and allergic diseases was observed.
- (5) The mean levels of serum albumin were significantly lower in the girls with the prevalence of asthma than those without.
- (6) The levels of serum  $\alpha_1$ AT,  $\alpha_2$ MG and

albumin were not associated with the intensity of exposure to ETS.

- (7) These findings suggest that serum  $\alpha_1$ AT and albumin levels are associated with respiratory and allergic diseases in children. A decreased  $\alpha_1$ AT level should be considered as a biological risk marker for these diseases.

### 要 旨

欧米では呼吸器疾患の危険因子として血清プロテアーゼ・インヒビターの欠損が知られているが、日本人における意義は明らかではない。日本の児童における血清プロテアーゼ・インヒビター及びアルブミン濃度と呼吸器症状との関連について検討するため、千葉県君津市の3小学校の4～3年生(1992年度)を対象に1992～1995年の4年間の追跡調査を行った。呼吸器・アレルギー症状と受動喫煙への曝露状況は年1回質問票調査により評価し、1992, 1994, 1995年には採血を行い、血清中 $\alpha_1$ -アンチトリプシン( $\alpha_1$ AT)、 $\alpha_2$ -マクログロブリン( $\alpha_2$ MG)及びアルブミン濃度を測定した。本研究では、1995年の質問票調査及び血液検査の結果が得られた652名(男子327名, 女子325名)を対象に解析を行った。血清 $\alpha_1$ AT及び $\alpha_2$ MG濃度は、年齢により変動が認められた。1992年に血清 $\alpha_1$ AT濃度が低値であったものは、1995年の喘息、喘鳴症状及びアレルギー疾患を有する割合が有意に高かった。血清 $\alpha_2$ MG濃度と喘息、喘鳴、アレルギー疾患との関連はみられなかった。1992年に喘息症状を有した女兒の血清アルブミン濃度は、症状のない女兒に比べて有意に低かった。血清 $\alpha_1$ AT及び $\alpha_2$ MG濃度と家庭内における受動喫煙への曝露との関連は認められなかった。以上より、児童の血清 $\alpha_1$ AT及びアルブミン濃度は、喘息、喘鳴症状及びアレルギー疾患との関連があることが示された。特に、血清 $\alpha_1$ AT濃度が低値であることは、その後の喘息とアレルギー疾患を予測する因子となる可能性があると考えられた。

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