

The age-adjusted cumulative incidence of diabetes was 9.6% for men and 5.9% for women, giving a statistically significant difference ($p = 0.002$). Figure 1 shows the age-adjusted cumulative incidence of diabetes according to quartiles of each liver enzyme level by sex. The cumulative incidence in the third and fourth GGT quartiles was significantly higher compared with that of the first quartile in both sexes. A similar tendency was observed for ALT quartiles: there were significant differences between the first and fourth quartiles in both sexes. This pattern was also found in AST quartiles for men but not for women.

The age-adjusted OR for the development of diabetes increased significantly with elevating quartiles of each liver enzyme concentrations in both sexes (Table 2, Model 1). In the multivariate analyses after adjustment for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, HDL-C, triglycerides, HS-CRP, hypertension, current drinking, current smoking, and physical activity, the ORs of future diabetes increased significantly with elevating quartiles of serum GGT and ALT (Model 2). These trends were also observed in AST quartiles for men but not for women. As shown in Model 3 of Table 2, after additional adjustment for the other liver enzymes, these relationships remained substantially unchanged in both GGT and ALT quartiles but not in AST in either sex.

To examine the influence of insulin resistance-related factors, inflammation and alcohol intake on the development of diabetes, we estimated the age- and sex-adjusted ORs and 95% CIs of diabetes by increments of 1 log in each liver enzyme in men and women together in accordance with other risk factor levels (Table 3). Analyses were performed dividing the subjects into three groups according to tertiles of fasting insulin, BMI, waist-to-hip ratio, and HS-CRP or to alcohol intake levels (0, 1 to 30, and ≥ 30 g/d). Significant positive associations of GGT and ALT with diabetes were observed in all stratified categories of each risk factor; however, we found no significant associations between AST and diabetes in the third tertile of BMI, in the third tertile of waist-to-hip ratio, or in the second level of alcohol intake.

To compare the ability of each risk factor to predict future diabetes over a mean of 9 years of follow-up, we plotted ROC curves and calculated optimal cut-off points, sensitivities, specificities, and the area under the ROC curves (Table 4). Both maximum sensitivity and specificity exceeded 60% only for GGT and ALT, and the areas under the ROC curve of GGT and ALT were significantly larger than that of AST, fasting insulin, waist-to-hip ratio, or HS-CRP and were slightly but not significantly larger than that of BMI. The difference in the area under the ROC curve between GGT and ALT was not significant.

Viral hepatitis infection can increase liver enzyme levels without liver fat accumulation. Thus, hepatitis B and C virus markers were examined in 1583 of the 1804 subjects in

1998. We found 13 viral hepatitis subjects (3 subjects with hepatitis B virus and 10 with C virus; 10.7%) in 122 subjects of the group developing diabetes and 104 viral hepatitis subjects (25 subjects with hepatitis B virus and 79 with C virus; 7.1%) in 1461 subjects of the group that did not develop diabetes: the difference was not significant ($\chi^2 = 2.1$; $p = 0.15$).

Discussion

We have shown, in a prospective study of a general Japanese population, that elevated levels of GGT and ALT, but not AST, are independent predictors of diabetes for both sexes after adjustment for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, HDL-C, triglycerides, HS-CRP, hypertension, current drinking, current smoking, physical activity, and the other liver enzymes. In our stratified analyses, associations of both GGT and ALT with the development of diabetes were observed in all layers of other risk factors, such as fasting insulin, BMI, waist-to-hip ratio, HS-CRP, and alcohol intake. ROC analyses showed that the predictive power of GGT and ALT was similar to that of BMI but stronger than that of AST, fasting insulin, waist-to-hip ratio, and HS-CRP. To the best of our knowledge, this study is the first report to indicate that liver enzymes are independent risk factors for developing diabetes in a general Japanese population in either sex, taking into account comprehensive risk factors for diabetes. Several prospective studies have found that high levels of hepatic enzymes, including GGT (2–6), ALT (7–9), and AST levels (10), are associated with later development of diabetes. These findings, together with these results, strongly suggest that the liver plays an important role in the development of diabetes in relatively lean Asian populations who may have smaller fat content in the liver, as it does in Western populations.

A recent study using a fatless mouse model has shown that ectopic fat accumulation in the liver is associated with severe insulin resistance (17). In normal weight and moderately overweight subjects, directly determined liver fat content has also been shown to correlate with several features of insulin resistance, independent of BMI and intra-abdominal or overall obesity (18). These findings indicate that hepatic fat accumulation is a critical manifestation of insulin resistance. However, direct measurement of liver fat requires ultrasound, computed tomography, or proton spectroscopy, and such techniques are unlikely to be recommended in routine clinical practice. Some circulating variables, including serum ALT, GGT, and AST, provide insight into the extent of liver fat accumulation. Among these, ALT is found primarily in the liver, whereas AST and GGT are also found in other tissues and are, therefore, less specific markers of liver function. Therefore, ALT is the most specific marker of liver pathology and seems to be the best marker for liver fat accumulation: serum ALT is cor-

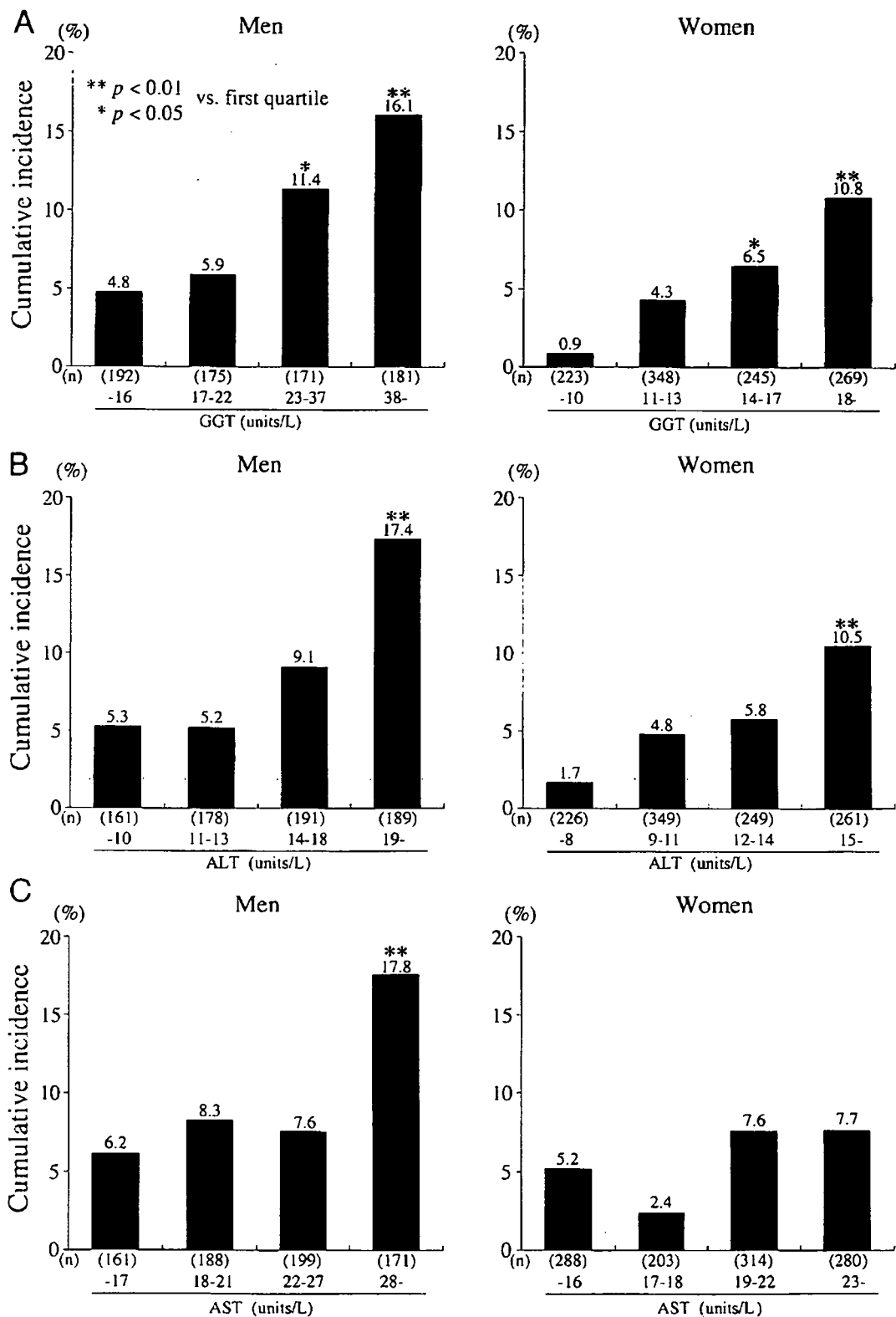


Figure 1: The age-adjusted cumulative incidences of diabetes according to quartiles of serum liver enzymes. (A) GGT, γ -glutamyltransferase; (B) ALT, alanine aminotransferase; (C) AST, aspartate aminotransferase.

Table 2. Age- and multivariate-adjusted ORs and 95% CIs for the development of diabetes according to quartiles of each liver enzyme by sex during mean 9 years of follow-up

	Range	Population at risk (n)	Number of events (n)	Model 1: OR (95% CI)	p for trend	Model 2: OR (95% CI)	p for trend	Model 3: OR (95% CI)	p for trend
GGT (U/L)									
Men	0 to 16	192	10	1 (referent)		1 (referent)		1 (referent)	
	17 to 22	175	10	1.10 (0.45 to 2.71)		0.85 (0.32 to 2.28)		0.85 (0.31 to 2.27)	
	23 to 37	171	20	2.39 (1.09 to 5.28)		2.02 (0.84 to 4.88)		1.99 (0.82 to 4.80)	
	38 to	181	31	3.71 (1.75 to 7.87)	0.0001	2.71 (1.13 to 6.52)	0.0040	2.54 (1.03 to 6.26)	0.0088
Women	0 to 10	223	3	1 (referent)		1 (referent)		1 (referent)	
	11 to 13	348	15	3.29 (0.94 to 11.48)		2.65 (0.74 to 9.47)		2.64 (0.74 to 9.42)	
	14 to 17	245	16	5.10 (1.46 to 17.74)		3.72 (1.04 to 13.29)		3.66 (1.02 to 13.09)	
	18 to	269	30	8.98 (2.70 to 29.87)	0.0001	5.80 (1.67 to 20.12)	0.0011	5.73 (1.62 to 20.19)	0.0017
ALT (U/L)									
Men	0 to 10	161	9	1 (referent)		1 (referent)		1 (referent)	
	11 to 13	178	9	0.90 (0.35 to 2.33)		0.71 (0.25 to 1.98)		0.68 (0.24 to 1.92)	
	14 to 18	191	17	1.65 (0.71 to 3.83)		1.31 (0.52 to 3.32)		1.18 (0.46 to 3.03)	
	19 to	189	36	3.98 (1.83 to 8.64)	0.0001	2.85 (1.17 to 6.92)	0.0017	2.32 (0.91 to 5.92)	0.016
Women	0 to 8	226	5	1 (referent)		1 (referent)		1 (referent)	
	9 to 11	349	17	2.18 (0.79 to 6.00)		2.28 (0.74 to 7.02)		2.26 (0.73 to 6.99)	
	12 to 14	249	14	2.60 (0.92 to 7.34)		2.83 (0.90 to 8.92)		2.86 (0.90 to 9.07)	
	15 to	261	28	5.15 (1.95 to 13.59)	0.0001	4.53 (1.50 to 13.64)	0.0027	4.40 (1.38 to 14.06)	0.0077
AST (U/L)									
Men	0 to 17	161	10	1 (referent)		1 (referent)		1 (referent)	
	18 to 21	188	16	1.43 (0.63 to 3.24)		0.96 (0.40 to 2.31)		0.91 (0.38 to 2.19)	
	22 to 27	199	15	1.26 (0.55 to 2.89)		0.88 (0.37 to 2.10)		0.81 (0.33 to 1.95)	
	28 to	171	30	3.27 (1.54 to 6.94)	0.0016	2.30 (1.01 to 5.21)	0.030	1.87 (0.77 to 4.53)	0.17
Women	0 to 16	288	12	1 (referent)		1 (referent)		1 (referent)	
	17 to 18	203	5	0.56 (0.19 to 1.62)		0.40 (0.12 to 1.29)		0.40 (0.12 to 1.28)	
	19 to 22	314	24	1.79 (0.86 to 3.73)		1.69 (0.80 to 3.58)		1.70 (0.79 to 3.62)	
	23 to	280	23	1.91 (0.91 to 4.04)	0.019	1.49 (0.68 to 3.24)	0.073	1.26 (0.55 to 2.92)	0.17

OR, odds ratio; CI, confidence interval; GGT, γ -glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Model 1: adjustment was made for age. Model 2: adjustment was made for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, high-sensitivity C-reactive protein, hypertension, current drinking, current smoking, and physical activity. Model 3: adjustment was made for the variables used in Model 2 and for the other liver enzymes

Table 3. Age- and sex-adjusted ORs and 95% CIs for the occurrence of diabetes by increments of 1 log in each liver enzyme according to risk factor levels during mean of 9 years of follow-up

	Range	Population at risk (n)	Number of events (n)	Age- and sex-adjusted		Age- and sex-adjusted		
				[OR (95% CI) for GGT]	p	[OR (95% CI) for ALT]	p	
Fasting insulin (pM)	0 to 24.0	605	32	2.93 (1.87 to 4.59)	0.0001	2.78 (1.45 to 5.35)	0.0022	
	24.1 to 36.0	547	36	2.21 (1.38 to 3.52)	0.0009	2.53 (1.39 to 4.63)	0.0025	
	36.1 to	651	67	1.86 (1.22 to 2.82)	0.0039	2.07 (1.31 to 3.26)	0.0018	
BMI (kg/m ²)	0 to 21.5	601	29	2.91 (1.82 to 4.64)	0.0001	2.87 (1.50 to 5.48)	0.0014	
	21.6 to 24.2	602	36	2.09 (1.30 to 3.38)	0.0025	3.43 (1.81 to 6.49)	0.0002	
	24.3 to	601	70	1.99 (1.32 to 3.00)	0.0010	1.71 (1.10 to 2.65)	0.016	
Waist-to-hip ratio	0 to 0.88	586	24	3.71 (2.10 to 6.54)	0.0001	2.19 (1.15 to 4.17)	0.017	
	0.89 to 0.94	583	59	2.28 (1.55 to 3.35)	0.0001	3.19 (1.86 to 5.49)	0.0001	
	0.95 to	590	50	1.78 (1.15 to 2.75)	0.01	2.24 (1.37 to 3.67)	0.0014	
HS-CRP (mg/L)	0 to 0.25	586	21	2.12 (1.21 to 3.73)	0.009	2.49 (1.31 to 4.74)	0.0056	
	0.26 to 0.64	587	46	2.62 (1.60 to 4.27)	0.0001	2.78 (1.50 to 5.15)	0.0011	
	0.65 to	586	64	2.09 (1.45 to 3.02)	0.0001	2.51 (1.59 to 3.96)	0.0001	
Alcohol intake (g/day)	0	1,274	79	2.81 (1.90 to 4.15)	0.0001	2.56 (1.73 to 3.79)	0.0001	
	1 to 29	289	24	2.86 (1.50 to 5.44)	0.001	3.04 (1.45 to 6.35)	0.0032	
	30 to	241	32	1.69 (1.08 to 2.64)	0.02	2.49 (1.24 to 5.00)	0.011	
							2.31 (1.12 to 4.74)	0.023

OR, odds ratio; CI, confidence interval; GGT, γ -glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-CRP, high-sensitivity C-reactive protein.

Table 4. Optimal cut-off points of risk factors defined by maximizing sensitivity and specificity to predict future diabetes and their ROC curve areas

	GGT (units/L)	ALT (units/L)	AST (units/L)	Fasting insulin (pM)	BMI (kg/m ²)	Waist-to- hip ratio	HS-CRP (mg/L)
Cut-off point	18	13	19	30.0	24.1	0.91	0.44
Sensitivity (%)	63.3	63.4	48.6	50.7	66.2	47.8	54.8
Specificity (%)	63.7	65.9	71.1	65.2	56.3	69.9	67.9
ROC curve area (%)	67.9	67.4	62.3*†	61.1*†	64.6	60.0*†	61.6*†
(95% CI)	(63.1 to 72.3)	(62.4 to 72.1)	(57.0 to 67.4)	(56.0 to 65.9)	(59.5 to 69.6)	(55.4 to 64.5)	(56.9 to 66.2)

ROC, receiver operating characteristic; GGT, γ -glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-CRP, high-sensitivity C-reactive protein; CI, confidence interval.

* $p < 0.05$ vs. GGT.

† $p < 0.05$ vs. ALT.

related with liver fat measured by proton spectroscopy and, after weight loss, the change in serum ALT correlates with that in liver fat (19).

In our multivariate analysis, serum GGT and ALT were mutually independent in predicting incident diabetes. It is known that serum GGT is not only a marker of liver fat amount but also a marker of oxidative stress (20–22). GGT presenting at the outer side of the cell membrane is thought to maintain cellular glutathione levels, which are the major intracellular defense against free radicals (23). Increased oxidative stress impairs insulin secretion from the islets of Langerhans and insulin action in target tissues by damaging DNA, membranes, enzymes, etc. (24). Decreased insulin secretion and insulin sensitivity are major features of the pathophysiology of type 2 diabetes (25). This may be the reason why GGT and ALT has a highly predictive value for the development of diabetes. On the other hand, several epidemiologic studies examined which of these enzymes was the best marker for incident diabetes. Lee et al. (3,4) reported the dose–response relationship between GGT levels and incidence of diabetes in both Korean male workers and young black and white Americans with ALT or AST levels within the reference interval. Furthermore, in their other study, GGT levels within normal range predicted incidence of chronic elevation of ALT (26). These findings indicate the possibility that GGT is a more powerful predictor of incident diabetes than other liver enzymes. However, we showed in the ROC analysis that ALT and GGT but not AST have equally predictive value for the development of diabetes. These findings should be confirmed in other populations, having different BMI levels and lifestyles.

Some experimental studies have shown that selective deletion of the insulin receptor from muscle results in a

slight increase in serum free fatty acid and triglycerides but not in glucose intolerance or diabetes (27), whereas a similar maneuver in the liver leads to marked glucose tolerance (28), suggesting that maintaining normal glucose concentrations is related to the liver rather than to peripheral tissue. Our stratified analysis showed that the associations of both GGT and ALT levels with the occurrence of diabetes were independent of markers of systemic insulin resistance, such as fasting insulin, BMI, waist-to-hip ratio, and HS-CRP. In our subjects, the areas under the ROC curve of GGT and ALT were also significantly larger than that of fasting insulin, waist-to-hip ratio, or HS-CRP. Insulin resistance in the liver through fat accumulation may offer a better explanation of the cause of diabetes than peripheral insulin resistance or systemic inflammation.

Alcohol intake causes fatty change of the liver. In alcoholic fatty liver, serum ALT tends to be depressed relative to serum AST, and serum GGT has the specificity to detect alcohol abuse (29), whereas liver fat accumulation caused by overeating predominantly increases ALT but not AST or GGT (19). However, these findings indicated that both GGT and ALT predict future diabetes, independent of current drinking habits. Additionally, in our stratified analyses, the associations of both GGT and ALT with diabetes were unrelated to alcohol intake levels. These observations suggest that elevated serum levels of GGT and ALT, irrespective of the causes of fatty liver, are associated with incident diabetes.

Viral hepatitis infection often increases liver enzyme levels without hepatic fat accumulation, and several clinical studies have shown that chronic hepatitis C virus infection is linked to type 2 diabetes (30,31). In our study, however, the distribution of hepatitis B and C virus positive markers

did not differ between subjects who developed diabetes and those who did not, indicating that viral hepatitis infection did not affect our findings.

A limitation of our study is that a diagnosis of diabetes was not based on a 75-gram oral glucose tolerance test, but on a single reading of fasting glucose level, as has been the case in other epidemiologic studies (3–5,9). Thus, subjects with diabetes having normal fasting glucose levels were misdiagnosed in our study. In addition, some of the participants who were classified as having worsening fasting glucose status may not have been so categorized after repeated testing. These misclassifications may have weakened the associations found in this study, and the true associations may, in fact, be stronger than those shown in our data.

In conclusion, we have shown that elevated serum GGT and ALT levels, even in the normal range, are better predictors of diabetes than the known risk factors except for BMI in a general Japanese population. The association between these enzymes and diabetes was found to be independent of insulin resistance, inflammation markers, and alcohol consumption levels. These results support the hypothesis that the liver is more important than previously thought in the pathogenesis of type 2 diabetes.

Acknowledgments

This study was supported, in part, by a Grant-in-Aid for Scientific Research C (13670370), a Special Coordination Fund for Promoting Science, and a Fund for Technology and Innovative Development Project in Life Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Albuminuria and Chronic Kidney Disease in Association With the Metabolic Syndrome

Chronic kidney disease (CKD) is a worldwide public health problem. CKD is a major risk factor for end-stage renal and cardiovascular disease and causes premature death.¹⁻⁵ CKD is also increasingly common. According to data from the Third National Health and Nutrition Examination Survey (NHANES III), approximately 8 million US adults 20 years of age and older currently have CKD. Therefore, identifying and treating risk factors for early CKD may be the best approach to prevent and delay advanced outcomes.²

The metabolic syndrome (MetS), previously known as syndrome X, the deadly quartet, and the insulin-resistant syndrome, is characterized by the cluster of atherogenic metabolic disorders, including abdominal obesity, high blood pressure, impaired glucose tolerance, hypertriglyceridemia, and reduced high-density lipoprotein cholesterol.⁶ The MetS is present in approximately 47 million US adults 20 years of age or older, according to data from NHANES III.⁷ With the continuous increase in the prevalence of obesity in the United States, the MetS is expected to be even more common in the future.⁸ In addition, the MetS has been associated with increased risk of developing diabetes mellitus and cardiovascular disease as well as increased mortality from cardiovascular disease and all causes.^{9,10} Recent epidemiologic surveys have found that patients with the MetS are at high risk for microalbuminuria or CKD. The aim of this article is to review the MetS as a risk factor for the development of microalbuminuria or CKD.

The MetS and Risk for Developing Renal Impairment

As shown in the Table, several epidemiologic studies examined the association between the MetS and the risk of microalbuminuria or CKD.¹¹⁻¹⁸ In

Chronic kidney disease is a worldwide public health problem because it is an important risk factor for cardiovascular disease and premature death. The metabolic syndrome, which is characterized by abdominal obesity, high blood pressure, impaired glucose tolerance, and dyslipidemia, is also an increasingly common disorder and a major risk factor for diabetes and cardiovascular disease. A close association has been found between the metabolic syndrome and the risk for developing renal impairment, clinically expressed in the form of microalbuminuria or chronic kidney disease. Several potential mechanisms, including insulin resistance, renal atherosclerosis, and inflammation, induce the deterioration of renal function. Despite the close association between the metabolic syndrome and renal impairment, it is still unclear whether and to what extent treating the metabolic syndrome will prevent renal impairment. A clinical trial is needed to clarify whether the effect of preventing and treating metabolic syndrome components will result in improved renal prognosis. (JCMS. 2007;2:104-107) ©2007 Le Jacq

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Manuscript received December 5, 2006; accepted January 15, 2007

these studies, microalbuminuria was defined as a urinary albumin/creatinine ratio of 30 to 300 mg/g, and CKD was determined by estimated glomerular filtration rate (GFR) <60 mL/min/1.73 m². GFR was estimated by the Modification of Diet in Renal Disease study equation.¹⁹

In a cross-sectional study of nondiabetic Native Americans, Hoehner and colleagues¹¹ found a significant association between MetS profile and microalbuminuria: subjects with 3 or more MetS components had a 2.3-fold higher prevalence of microalbuminuria than did those with no components after adjustment for social, demographic, and comorbid factors. Palaniappan and associates¹² and Chen and colleagues¹³ extracted data from NHANES III, which contained detailed clinical information on approximately 6000 subjects. Both of these cross-sectional studies

showed significant associations between the MetS, defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria, and microalbuminuria. Chen and colleagues also demonstrated that subjects with the MetS had a 2.6-fold higher prevalence of CKD compared with patients without the MetS, and the prevalence of CKD increased with elevating number of MetS components.¹³ In Asian populations, a few studies also examined this issue.^{14,15} In a cross-sectional survey of Korean adults who participated in a health examination program, the multivariate-adjusted odds ratio of microalbuminuria in subjects with the MetS was 1.5 compared with patients without the MetS.¹⁴ The similar finding was observed in the results of health checks in Okinawa, a southern island in Japan.¹⁵ Although the results of these studies suggest a close association

Table. Epidemiologic Studies Concerning the Association Between the MetS and CKD

AUTHORS	COUNTRY	NO. OF SUBJECTS	DESIGN	METS DEFINITION	END POINT	MULTIVARIATE-ADJUSTED OR (95% CI)
Hoehner et al ¹¹	United States	2068	Cross-sectional	Original ^a	MAU	2.3 (1.1–4.9)
Palaniappan et al ¹²	United States	5659	Cross-sectional	NCEP	MAU	Men: 4.1 (2.5–6.7) ^b Women: 2.2 (1.4–3.3) ^b
Chen et al ¹³	United States	6217	Cross-sectional	NCEP	MAU	CKD MAU: 1.9 (1.3–2.7) CKD: 2.6 (1.7–4.0)
Choi et al ¹⁴	Korea	6588	Cross-sectional	NCEP ^c	MAU	1.5 (1.1–2.1)
Tanaka et al ¹⁵	Japan	6980	Cross-sectional	NCEP ^d	CKD	1.5 (1.3–1.9)
Kurella et al ¹⁶	United States	10,096	Prospective	NCEP	CKD	1.4 (1.2–1.7)
Ninomiya et al ¹⁷	Japan	1440	Prospective	NCEP ^c	CKD	2.1 (1.2–3.5)
Bonnet et al ¹⁸	France	2738	Prospective	NCEP IDF	MAU	NCEP: Men: 1.2 (0.7–2.2) Women: 1.9 (0.9–3.8) IDF: Men: 1.9 (1.3–2.8) Women: 1.4 (0.8–2.5)

Abbreviations: NCEP, National Cholesterol Education Program; IDF, International Diabetes Federation. ^aThe metabolic syndrome (MetS) was defined by hypertension, impaired fasting glucose, high fasting insulin, low high-density lipoprotein cholesterol, and hypertriglyceridemia. ^bAge-adjusted odds ratio (OR) and 95% confidence intervals (CIs). ^cWaist circumference was modified by the Asian criteria of waist circumference (men >90 cm, women >80 cm). ^dWaist circumference was modified by the Japanese criteria of waist circumference (men >85 cm, women >90 cm). Microalbuminuria (MAU) was defined as a urinary albumin/creatinine ratio of 30 to 300 mg/g. Chronic kidney disease (CKD) was defined as glomerular filtration rate estimated by Modification of Diet in Renal Disease study equation <60 mL/min/1.73 m².

between the MetS and renal impairment, it is difficult to draw any definitive conclusion concerning a cause-and-effect relationship because these studies had a cross-sectional design.

Recent longitudinal epidemiologic studies provide further evidence to support the premise that the MetS independently contributes to the development of microalbuminuria or CKD.^{16–18} Kurella and colleagues¹⁶ showed in a 9-year follow-up survey of the Atherosclerosis Risk in Communities (ARIC) study that the MetS was independently associated with increased risk of incident CKD in nondiabetic adults; the risk for the development of CKD was 1.4-fold higher in subjects with the MetS than in patients without the MetS after adjustment for potential confounding factors. We also found that the MetS was an independent risk factor for incident CKD in a 5-year follow-up survey of the Hisayama Study,¹⁷ a prospective cohort study of cardiovascular disease and its risk factors in a general Japanese population. Subjects with the MetS had a 2.1-fold increased risk for incident CKD compared with patients without the MetS after multivariate adjustment.

Even after exclusion of subjects with diabetes at entry or adjustment for blood pressure at end of follow-up, the risk for incident CKD in subjects with the MetS remains significant. In a 6-year follow-up survey of the Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) trial, Bonnet and colleagues¹⁸ showed that in men, the MetS was a significant risk factor for developing microalbuminuria according to the International Diabetes Federation (but not the NCEP-ATP III) definition of MetS. The presence of the MetS according to either definition was not related to the development of microalbuminuria in women.

These studies suggest the strong evidence that the MetS is an independent risk factor for developing microalbuminuria or CKD. A further clinical trial is needed to clarify whether the effect of preventing and treating the MetS will result in improved renal prognosis.

Mechanism of Developing Renal Impairment in the MetS

Several possible explanations have been proposed for the mechanism underlying the association between the MetS

and the risk of renal impairment: (1) the adverse effects of insulin resistance, (2) the accumulation of atherogenic risk factors, (3) the adverse effects of inflammatory mediators, and (4) obesity-related glomerulopathy.

Insulin Resistance. Insulin resistance and compensatory hyperinsulinemia, which are considered to be fundamental pathogenetic factors of the MetS, directly contribute to the development of renal injury by worsening renal hemodynamics through multiple mechanisms, including sodium retention,²⁰ activation of the sympathetic nervous system,²¹ decreased Na⁺-K⁺-ATPase activity,²² and elevation of the glomerular filtration fraction.²⁰ In addition, on the basis of in vitro observations, hyperinsulinemia could induce glomerular hypertrophy either directly or by stimulating the insulin-like growth factor 1 (IGF-1) receptor.²³ Moreover, treatment of mesangial cells with insulin resulted in a marked increase in rate of protein synthesis as well as in an alteration in the type of interstitial and basement membrane collagen excreted by these cells.^{24,25} Interestingly, insulin also seemed to evoke a phenotypic

change in mesangial cells, and these cells failed to synthesize a normal collagen pattern, namely, predominant synthesis of collagen type I and III, instead of collagen IV.^{24,25} IGF-1 was found to inhibit apoptosis of mesangial cells in a dose-dependent manner, thereby promoting their survival.²⁶ IGF-1 also decreased the activity of matrix metalloproteinase-2, an enzyme normally responsible for extracellular matrix degradation; its inhibition led to extracellular matrix expansion and renal fibrosis.²⁷ Hyperinsulinemia could further interact with elevated intrarenal angiotensin II levels to augment angiotensin II concentration of glomerular mesangial cells.²⁰

Atherogenic Risk Factors. Another possible explanation for the relationship between the MetS and incident CKD is that MetS components directly damage the kidneys through atherosclerosis. The mechanisms of hypertensive and diabetic renal injuries leading to CKD have been well described.²⁸⁻³⁰ In the ARIC study, however, the association between the MetS and CKD in nondiabetic subjects remained robust even after adjustment for the subsequent development of diabetes and hypertension, suggesting that the risk for CKD is not solely attributable to the conditions of diabetes or hypertension.¹⁶ In NHANES III, Chen and colleagues observed that increased abdominal obesity was significantly correlated with microalbuminuria and GFR decline, suggesting that obesity may be an independent risk factor for CKD.¹³ Observational data and a recent meta-analysis suggest that elevated triglycerides and reduced high-density lipoprotein cholesterol are independent risk factors for the development of CKD and that the use of statins may slow CKD progression.³⁰⁻³² In our autopsy-based study of Hisayama residents,³³ hypertension, impaired glucose tolerance, and hypercholesterolemia were also significant risk factors for renal arteriosclerosis. Although data from the ARIC study also showed that each MetS component was significantly

associated with increased risk for incident CKD, the cluster of these risk factors had a stronger impact on the development of CKD than did individual risk factors.¹⁶ Furthermore, we observed in the Hisayama Study that the accumulation of 3 or more of the metabolic disorders promoted the development of CKD or progression of GFR decline independent of serum fasting insulin levels. This suggests that the risk of cluster of MetS components for the development of CKD is not solely attributable to the insulin resistance and compensatory hyperinsulinemia.¹⁷ These findings support the hypothesis that the accumulation of atherogenic metabolic disorders induces renal vessel injury, resulting in the deterioration of renal function.

Inflammatory Mediators and Obesity-Related Glomerulopathy. Another potential mechanism includes direct effects of inflammatory mediators resulting from obesity and insulin resistance.³⁴ Adipose tissue is recognized as an immune organ that secretes numerous immunomodulatory factors, including leptin, interleukin 6, tumor necrosis factor α , adiponectin, and acylation-stimulation protein.³⁴ These adipose tissue cytokines may be crucial to the enhancement of insulin resistance or systemic atherosclerosis, resulting in the progression of CKD. Furthermore, a large renal biopsy-based clinicopathologic study demonstrated that the frequency of obesity-related glomerulopathy, which is characterized by focal segmental glomerulosclerosis and glomerulomegaly, increased from 0.2% to 2% in the patients who received renal biopsy during 15 years of the study period.³⁵ This glomerulopathy may also contribute to the renal dysfunction.

Potential Strategies for Preventing Renal Impairment in the MetS

The observed association between the MetS and the risk of developing CKD raises the question of whether correcting 1 or more of the syndrome's features may effectively prevent CKD.

It has been shown that intensive blood pressure and blood glucose control effectively prevents the development of microalbuminuria and CKD in diabetic patients.³⁶ Furthermore, the nephroprotective effects of angiotensin-converting enzyme inhibitors have been suggested by a number of clinical trials involving diabetic and nondiabetic patients.^{37,38} In addition, recent studies showed the effectiveness of lipid-lowering treatments in decreasing albuminuria and slowing the rate of decline in GFR in patients with CKD.^{39,40} There are no clinical trials, however, that address whether treating MetS components will reduce the risk of development or progression of renal impairment; neither are there trials that define optimum target levels of treated components in these settings. Finally, although there is no doubt that all obese subjects should be encouraged to undertake physical activity and change their eating habits, further studies should address whether weight reduction, exercise, and other measures to increase insulin sensitivity as well as interventions that directly target biochemical MetS components, may reduce the risk for microalbuminuria or CKD. Until such trials are conducted, the only available preventive strategy consists of considering patients with the MetS as a subset of patients who are at high risk for developing microalbuminuria or CKD and who therefore require close monitoring to ensure the early recognition of subsequent renal impairment and aggressive treatment of all metabolic alterations.

Conclusions

A close association has been found between the MetS and the risk of developing renal impairment, clinically expressed in the form of microalbuminuria or CKD. This finding raises a major clinical and public health concern because both the MetS and CKD are increasingly common disorders. Several potential mechanisms, including insulin resistance, renal atherosclerosis, or inflammatory mediators, induce renal injury, resulting in the deterioration of renal function.

Despite the close association between the MetS and renal impairment, it is still unclear whether and to what extent

treating patients with the MetS will prevent the development and progression of CKD. A clinical trial is needed

to clarify whether the effect of preventing and treating MetS components will result in improved renal prognosis.

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Altered Expression of COX-2 in Subdivisions of the Hippocampus during Aging and in Alzheimer's Disease: The Hisayama Study

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Key Words

Cyclooxygenase · Alzheimer's disease · Hippocampus

Abstract

Background: It has been reported that nonsteroidal anti-inflammatory drugs may delay the onset of Alzheimer's disease (AD). Since nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase (COX), COX-2, an inducible form of COX, may be involved in the pathology of AD in association with the arachidonic acid cascade. In addition, it has been suggested that alterations in the balance of polyunsaturated fatty acids are associated with brain dysfunctions such as neurodegenerative pathologies of the aging brain. **Method:** To explore COX-2 expression in the hippocampus, we analyzed 45 consecutive autopsy subjects without dementia and 25 AD patients derived from the town of Hisayama, Japan. **Results:** The neuronal expression of COX-2 in the CA3 subdivision of the hippocampus, subiculum, entorhinal cortex and transentorhinal cortex were consistently observed in both nondemented and AD brains, and COX-2 immunoreactivity correlated with age in nondemented brains. In AD patients, neurons of CA1 exhibited increased COX-2 immunoreactivity which correlated with the severity of AD pathology. This correlation was not apparent in nondemented subjects. **Conclusion:** These results suggest that COX-2 expression may be

differentially regulated among subdivisions of the hippocampus and that elevated COX-2 expression in the CA1 of AD brains may be associated with AD pathology and thus cognitive dysfunction.

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Introduction

Many epidemiological studies suggest that the use of nonsteroidal anti-inflammatory drugs delays or slows the clinical expression of Alzheimer's disease (AD) [1, 2]. The mechanism by which these drugs might affect pathophysiological processes relevant to AD remains unclear. Most nonsteroidal anti-inflammatory drugs have an inhibitory effect on cyclooxygenase (COX), an enzyme involved in the metabolism of arachidonic acid into prostanoids. There are two major known COX isoforms, the constitutively expressed COX-1 and the mitogen-inducible COX-2 [3]. While COX-1 is mainly expressed in microglia and some neuronal cells throughout the brain, COX-2 is expressed in neurons [4, 5]. It has been suggested that alterations in the balance of polyunsaturated fatty acids, including arachidonic acid and its metabolites, in the central nervous system are associated with brain dysfunction, such as in neurodegenerative pathologies of the aging

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1420-8008/07/0236-0423\$23.50/0

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brain [6]. Following on these epidemiological reports, several histological analyses of COX-2 expression in AD brains have been conducted [4, 7–11], but have produced conflicting results. Several studies reported increased neuronal COX-2 immunoreactivity compared to control brain tissues [5, 7]. However, in other studies, in which COX-2 expression was related to specific hallmarks of the disease, such as clinical dementia rating and Braak stage of disease, the number of COX-2-positive neurons decreased with the severity of dementia, and in the end-stage AD, COX-2-positive neurons were significantly fewer than in nondemented controls [4, 11].

Although many studies have been conducted concerning COX-2 expression not only in AD brains, but also in Parkinson disease model mice brains [12], amyotrophic lateral sclerosis brains [13] and schizophrenia brains [14], the histological analyses concerning COX-2 expression in nondemented brains are few. Now that we know that COX-2 expresses constitutively in the brain even under normal conditions [15], it is important to explore the normal COX-2 expression pattern in the brain. Without this basic knowledge, it is difficult to interpret the conflicting results of COX-2 expression in AD brains.

In this study, we investigated the neuronal expression of COX-2 in some subdivisions of hippocampi of consecutive autopsy cases without dementia. In addition, we quantified senile plaque (SP) and neurofibrillary tangle (NFT) density to assess the influence of AD pathology on neuronal COX-2 expression, and explored any differences in the pattern of COX-2 expression in these regions between nondemented subjects and AD patients.

Materials and Methods

Subjects

To minimize the selection bias of the nondemented subjects, we collected the nondemented subjects from the series of consecutive autopsy cases in the Hisayama study. The Hisayama study is a prospective population-based study in the suburban community of Hisayama, which is adjacent to the metropolitan area of Fukuoka on Kyushu Island, Japan, and it started in 1961 [16–19]. We have carried out autopsies on most deceased subjects from this region in order to confirm the cause of death and to examine brain pathology. This paradigm has allowed a reliable recruitment of nondemented subjects in which to analyze the neuronal expression of COX-2. The diagnosis of dementia was based on the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition [20]. For the clinical diagnosis of AD, we used the guidelines of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [21].

From October 1, 1998, to March 31, 2001, 148 Hisayama residents of varying initial ages died, 105 of whom (70.5%) underwent a postmortem examination. Consent to autopsy was unobtainable from 29.5% of the residents due to refusal, mainly on religious grounds. Of those 105 cases, 103 subjects received autopsies at the Departments of Pathophysiological and Experimental Pathology, Anatomic Pathology and Neuropathology of Kyushu University. In order to collect consecutive autopsy cases without dementia, we excluded 58 cases that were clinically diagnosed as exhibiting dementia or had some disease or condition that might influence the expression of COX-2 in the brain, such as severe chronic hepatic failure, autoimmune disease, disseminated intravascular coagulation, systemic inflammatory response syndrome, acute brain infarction, brain infection or a brain tumor. In total, 45 cases were analyzed in study A as nondemented subjects. In study B, in order to compare the nondemented subjects with AD patients, we examined all of the nondemented subjects aged 76 years or more at death of the nondemented subjects of study A and age- and sex-matched AD autopsy cases derived from Hisayama Town as the comparison group. In total, 25 nondemented subjects and 25 AD patients were analyzed in study B. We examined only cases aged 76 or more because there were few AD patients younger than 75 in the Hisayama study.

Neuropathological Assessment

Brains were weighed, evaluated for gross detectable lesions, abnormalities of the blood vessels and were fixed with 10% buffered formalin for at least 2 weeks. Brain specimens were taken following the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) guidelines and the consensus guidelines for dementia with Lewy bodies [22, 23]. Thus, the specimens in each case included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulate gyrus, hippocampus with entorhinal cortex and transentorhinal cortex (at the level of the lateral geniculate body), calcarine cortex, basal ganglia, thalamus, substantia nigra, locus coeruleus and dorsal vagal nucleus. Samples were embedded in paraffin and cut into sections which were routinely stained using hematoxylin-eosin and a modified Bielschowsky method. Each case was also immunostained with anti-tau (polyclonal, rabbit, 1:200, Dako, Denmark), anti-ubiquitin (polyclonal, rabbit, 1:100, Dako) and anti- α -synuclein (LB509: monoclonal, mouse, 1:100, provided by Dr. Iwamoto) [24]. Immunolabeling was detected using a standard indirect immunoperoxidase method and visualized with diaminobenzidine (Dojindo, Japan). The sections were lightly counterstained with hematoxylin.

Assessment of AD Pathology

The presence of SPs was estimated by a modified Bielschowsky method. NFT presence was assessed by tau immunohistochemistry. In each case, the frequency of SPs and NFTs were evaluated and converted to a plaque score according to CERAD criteria and Braak stage for tau pathology as established by Braak and Braak [22, 25]. The CERAD score and Braak stage were combined to estimate the likelihood of AD according to the NIA-RI criteria [26]. A diagnosis of AD was made when 'definite AD' as defined by the CERAD criteria and/or a 'high-likelihood' as defined by the NIA-RI criteria were found.

In addition, SP and NFT levels in the CA1 subdivision of the hippocampus were assessed. The semiquantitative density of SPs in

CA1 was determined as being either none, sparse, moderate, or frequent, according to the guidelines established by CERAD. NFTs in CA1 were counted in 100× fields at each of three locations and the average was expressed as the NFT number per 100× field.

Immunohistochemistry and Assessment of COX-2

Immunohistochemistry was performed on 7-μm paraffin-embedded sections encompassing the hippocampus, entorhinal cortex and transentorhinal cortex (at the level of the lateral geniculate body). Sections were deparaffinized in xylene, hydrated in an ascending ethanol series and incubated in 0.3% hydrogen peroxide in absolute methanol for 30 min at room temperature to inhibit endogenous peroxidase activity. After rinsing with tap water, the sections were pretreated with 90% formic acid for 10 min and autoclaved at 121°C for 10 min in 0.01 M citrate buffer, pH 6.0, in order to enhance immunoreactivity. After washing with Tris-HCl buffer (50 mM Tris-HCl, pH 7.6), an anti-human COX-2 (polyclonal, rabbit, 1:100, Cayman Chemical Co.) was applied. The slides were incubated overnight at 4°C and then sequentially incubated for 1 h with a biotinylated secondary antibody diluted 1:200, and a peroxidase-conjugated streptavidin-biotin complex diluted 1:100 sequentially (Amersham, UK). The colored reaction product was developed with 3,3'-diaminobenzidine tetrahydrochloride solution. The sections were then lightly counterstained with hematoxylin.

For analysis of neuronal COX-2 immunoreactivity, the mean gray values of a random-selected 5 neurons and 5 neuropil backgrounds were quantified using ImageJ 1.36b (National Institute of Health, USA) and the average was calculated. Then, we converted the mean gray value into a density using the following equation; uncalibrated density = $\log_{10}(255/\text{the mean gray value})$. Finally, we calculated the index that the neuronal density was divided by the neuropil background density, and we considered this index as the neuronal immunostaining density of COX-2. This index was calculated in CA1, CA3, subiculum entorhinal cortex and transentorhinal cortex.

The investigator was blind to the diagnosis of each case until analysis was completed and values were assigned to each specimen.

Statistical Methods

The quantitative data obtained were compared between the groups by Mann-Whitney's U test. Statistical significance was defined as $p < 0.05$. Correlation analysis was done using the Pearson parametric and Spearman nonparametric methods.

Results

Study A

Clinico-Neuropathological Information of Nondemented Subjects

The number of the nondemented subjects was 45 (M/F: 28/17). The ages at death were between 40 and 95 years and mean age at death was 76.4 ± 12.1 years. The brain weight was $1,264.0 \pm 166.8$ g (mean \pm SD) and postmortem time was 13.1 ± 9.3 h (mean \pm SD).

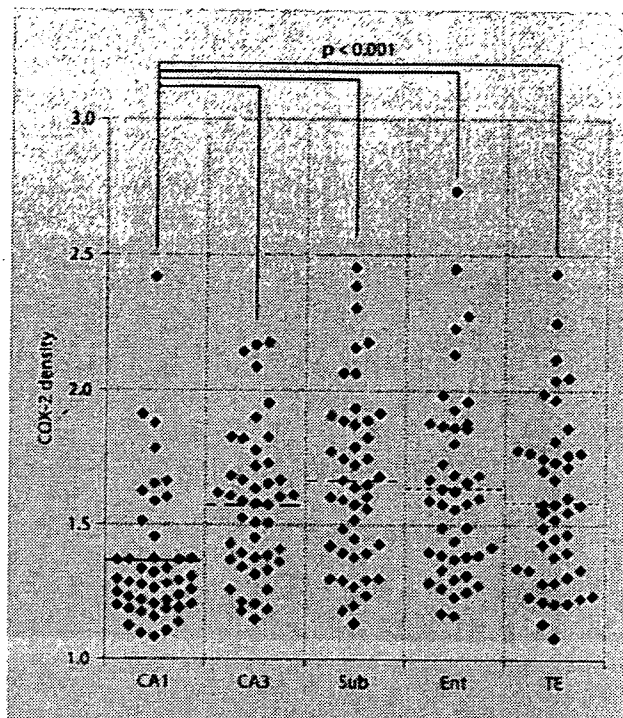


Fig. 1. Degrees of COX-2 immunoreactivity in different hippocampal subdivisions of nondemented subjects. Immunoreactivity is weak in CA1 as compared to all of the other fields examined with high statistical significance (Mann-Whitney U test, $p < 0.001$). Bars represent the mean density of neurons in each area. Sub = Subiculum; Ent = entorhinal cortex; TE = transentorhinal cortex.

COX-2 Immunoreactivity in the Hippocampus

The degree of COX-2 immunoreactivity in different hippocampal subdivisions of nondemented subjects is shown in figure 1. In nondemented subjects, the neuronal COX-2 immunoreactivity in CA3, subiculum, entorhinal cortex and transentorhinal cortex were strong (fig. 2a, d). On the other hand, the neuronal COX-2 immunoreactivity in CA1 was weak (fig. 2a, c) compared to all of the other fields examined, with high statistical significance (Mann-Whitney U test, $p < 0.001$). From these results, the constitutive expressions of COX-2 in CA3, subiculum, entorhinal cortex and transentorhinal cortex were thought to be strong, while weak in CA1.

COX-2 Immunoreactivity Correlates with Age in Nondemented Subjects

The correlation between neuronal COX-2 immunoreactivity and age is shown in figure 3. In the CA3 subdivi-

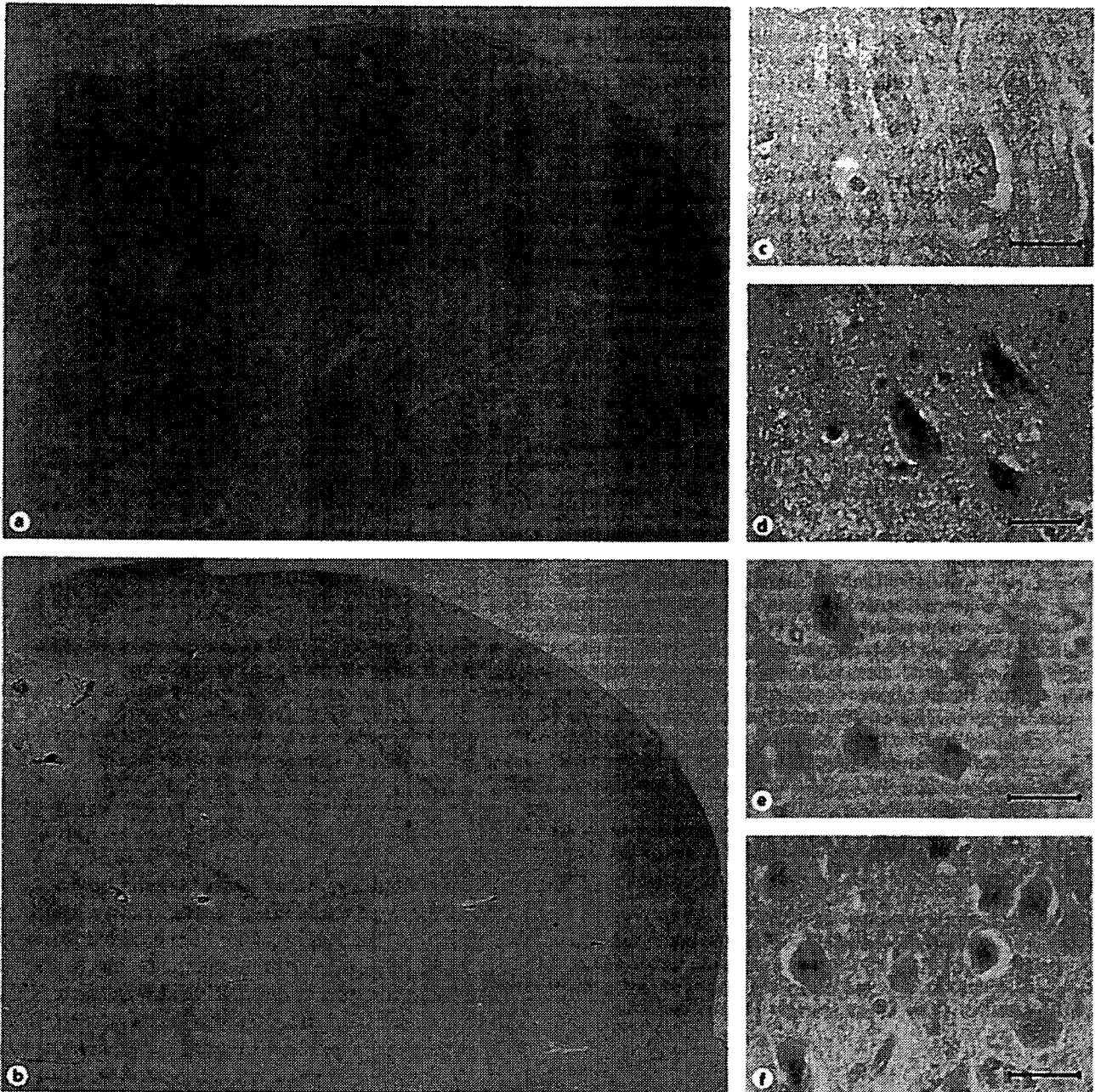


Fig. 2. COX-2 immunostaining in pyramidal neurons of the hippocampal formation in the brains of nondemented subjects (**a, c, d**) and in AD patients (**b, e, f**). **a, b** CA1-CA4 fields under low power. **c, e** Immunostaining within the CA1 field. **d, f** Immunostaining within CA3. Neuronal expression of COX-2 in CA3 is widely seen among both nondemented subjects and AD patients. Although neuronal expression of COX-2 in CA1 is widespread in AD patients, it is less detectable among nondemented subjects. **c-f** Bars are 30 μ m.

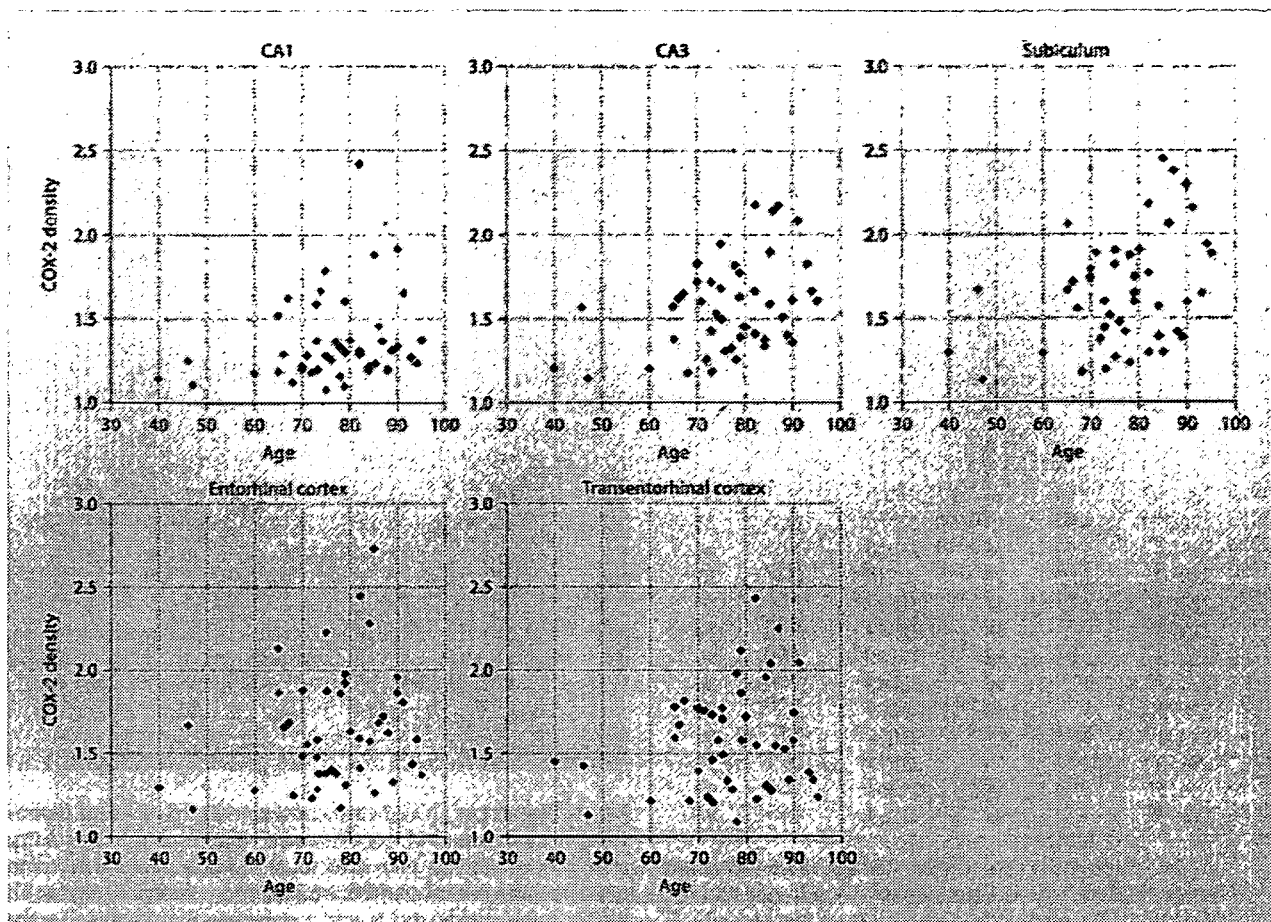


Fig. 3. A significant correlation exists between aging and COX-2 immunoreactivity in CA3 and the subiculum of nondemented subjects. In nondemented subjects, aging correlates with COX-2 immunoreactivity in CA3 and the subiculum (Pearson's correlation coefficient test, $r = 0.399$, $p = 0.007$; $r = 0.380$, $p = 0.010$, respectively).

sion of the hippocampus and subiculum of nondemented subjects, COX-2 immunoreactivity correlated with age (Pearson's correlation coefficient test, $r = 0.399$, $p = 0.007$; $r = 0.380$, $p = 0.010$, respectively) and this correlation was not evident in the CA1 subdivision of the hippocampus, entorhinal cortex or transentorhinal cortex (Pearson's correlation coefficient test, $r = 0.268$, $p = 0.078$; $r = 0.220$, $p = 0.147$; $r = 0.194$, $p = 0.202$, respectively).

Study B

Information of Nondemented Subjects and AD Patients

In study B, in order to compare the nondemented subjects with AD patients, we examined 25 nondemented subjects aged 76 years or more from study A and we col-

lected another 25 age- and sex-matched AD autopsy cases derived from Hisayama Town (table 1). All of the AD patients were free of other types of dementia.

COX-2 Immunoreactivity in the Hippocampus

The degrees of COX-2 immunoreactivity in different hippocampal subdivisions of nondemented subjects and AD patients are shown in figure 4. The immunoreactivity in CA1 was increased in AD patients as compared to nondemented subjects with high statistical significance (Mann-Whitney U test, $p = 0.001$; fig. 2c, e). On the other hand, the differences between the nondemented subjects and AD patients were small in CA3, subiculum, entorhinal cortex and transentorhinal cortex (Mann-Whitney U test, $p = 0.171$, $p = 0.467$, $p = 0.712$, $p = 0.621$,

Table 1. Subjects of study B

Nondemented subjects			Age- and sex-matched AD patients		
ID	Sex	Age	ID	Sex	Age
22735	F	76	23297	F	77
22907 ^C	F	77	21363	F	78
22880	F	78	22598	F	79
22939 ^C	M	78	23373	M	79
22739	F	79	23114	F	79
22910 ^C	F	79	23392	F	79
22819	M	79	20565	M	80
22803	M	80	23185	M	81
22933	M	82	20189	M	83
22828 ^C	F	82	20617	F	82
22892	M	82	20316	M	83
22772 ^C	F	84	20461	F	84
23015 ^C	F	84	22502	F	84
23061	M	85	20706	M	85
22906	F	85	23018	F	84
22950	F	86	20240	F	87
22795	M	87	21501	M	86
22976 ^C	F	88	23334	F	88
22798	M	89	22156	M	90
22767	F	90	20748	F	90
22992	F	90	23289	F	90
23021 ^C	M	91	23028	M	92
22955 ^C	F	93	23377	F	93
23055 ^C	F	94	22661	F	94
22896 ^C	F	95	23269	F	95

We studied all subjects aged >76 years of the 45 nondemented subjects of study A. As a comparison, we collected 25 age- and sex-matched AD patients. The subjects whose IDs are marked with 'C' are nondemented subjects with AD pathology (CERAD: moderate or frequent, with Braak and Braak stage 4, 5 or 6) and are the subjects of study C.

respectively). From these results, COX-2 immunoreactivity in CA1 has presumably been induced along with the development of AD; therefore, we assessed the influence of AD pathology on neuronal COX-2 expression in CA1.

COX-2 Immunoreactivity in CA1 Correlates with SP and NFT Density in AD Patients

In the CA1 subdivision of the hippocampi of AD patients, COX-2 immunoreactivity correlated with the semiquantification of SPs (Spearman's rank correlation test, $\rho = 0.500$, $p = 0.001$; fig. 5a) and with the number of NFTs (Pearson's correlation coefficient test, $r = 0.536$, $p = 0.003$; fig. 5b). On the other hand, in the CA1 subdivision of the hippocampi of nondemented subjects,

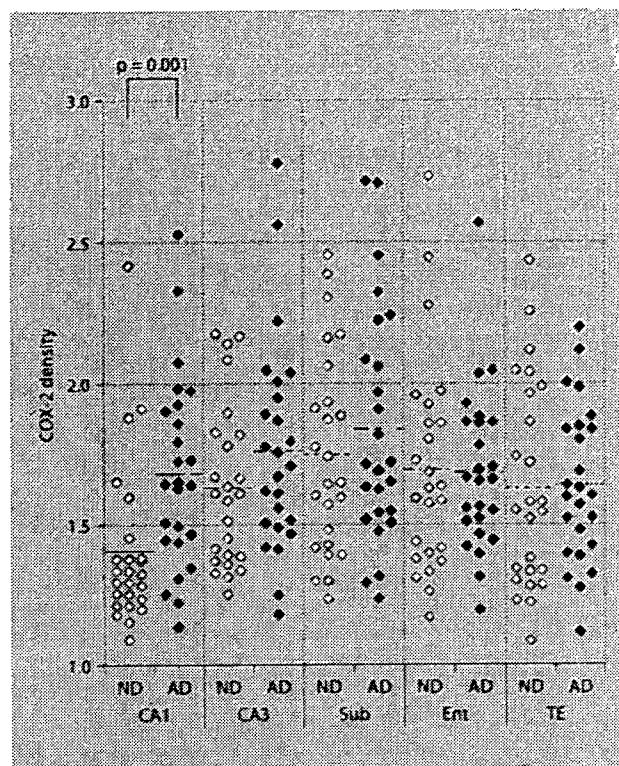


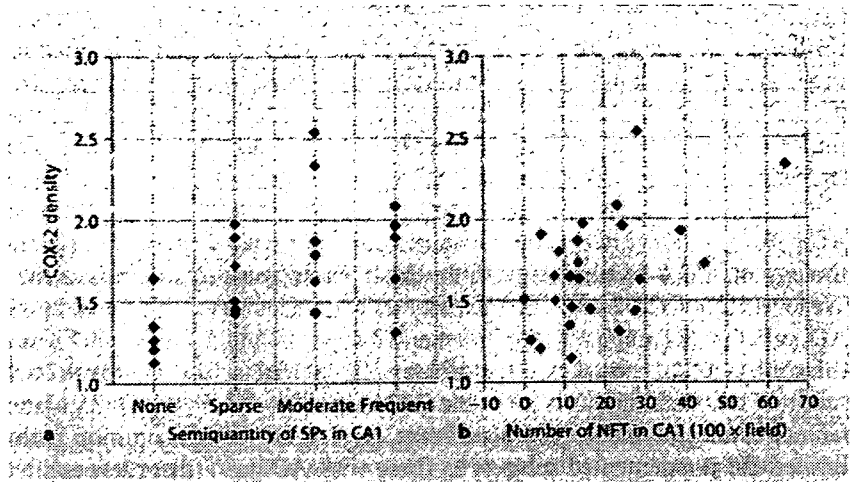
Fig. 4. Degrees of COX-2 immunoreactivity in different hippocampal subdivisions of nondemented subjects and AD patients. Immunoreactivity is increased in AD patients as compared to nondemented subjects, and the increase is observed in almost all hippocampal fields and reached statistical significance in the CA1 field (Mann-Whitney U test, $p = 0.001$). Bars represent the mean density of neurons in each area.

COX-2 immunoreactivity did not correlate with either the semiquantification of SPs (Spearman's rank correlation test, $\rho = 0.013$, $p = 0.949$) or the number of NFTs (Pearson's correlation coefficient test, $r = -0.141$, $p = 0.501$).

Study C

In the Hisayama study, we sometimes encountered autopsy cases that were cognitively normal though exhibited severe Alzheimer type pathology in their brains. Therefore, in study C, we compared the immunoreactivity of COX-2 in CA1 of 11 nondemented subjects with AD pathology (CERAD: moderate or frequent, with Braak and Braak stage 4, 5 or 6; table 1) with that of 11 age- and sex-matched AD patients. The immunoreactivity of COX-

Fig. 5. COX-2 immunoreactivity in CA1 correlates with SP (a) and NFT (b) density in AD patients. The semiquantitative density of SPs in CA1 was determined using guidelines established by CERAD. In AD patients, a correlation between COX-2 immunoreactivity and the semiquantitative density of SPs in CA1 was statistically significant (Spearman's rank correlation test, $\rho = 0.500$, $p = 0.001$). Also, a correlation between COX-2 immunoreactivity and the density of NFT in CA1 was statistically significant (Pearson's correlation coefficient test, $r = 0.536$, $p = 0.003$).



2 in CA1 of AD patients was obviously stronger than in nondemented subjects with AD pathology (Mann-Whitney U test, $p < 0.001$; fig. 6).

Discussion

To our knowledge, this is the first report that explores the regional distribution of COX-2 immunoreactivity in the hippocampi of nondemented subjects of a general population. We found that among nondemented subjects, COX-2 immunoreactivity in CA3, subiculum, entorhinal cortex and transentorhinal cortex was widespread, suggesting that COX-2 is constitutively expressed in these subdivisions of the hippocampus, whilst weak immunoreactivity was observed in CA1. In addition, in the CA3 subdivision of the hippocampi and subiculum of nondemented subjects, COX-2 immunoreactivity correlated with age, suggesting that the COX-2 expression in this region was augmented with aging in the normal condition.

Strong expression of COX-2 in the CA3 subdivision of the hippocampus and expression of COX-2 in the subiculum was reported previously in the normal rat brain [27]. Also, an immunohistochemical study of the human hippocampus showed more COX-2 positive neurons to be present in the CA3 than in the CA1 or CA2 field, and this difference became clearer when analysis was limited to dense staining [11]. These results are compatible with our data and indicate that our staining and assessment of COX-2 are reasonable and also suggest the constitutive expression of COX-2 in these subdivisions of the hippocampus.

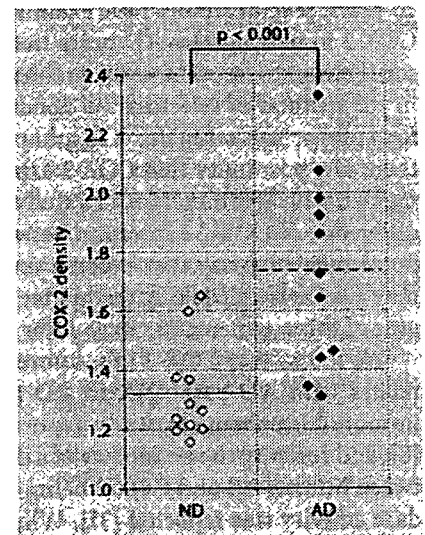


Fig. 6. Degrees of COX-2 immunoreactivity in the CA1 subdivision of the hippocampus of nondemented subjects with AD pathology and AD patients. COX-2 immunoreactivity is increased in AD patients as compared to nondemented subjects (Mann-Whitney U test, $p < 0.001$). Bars represent the mean density of neurons in each area.

The enhanced expression of COX-2 with age was reported in a study examining aged mouse macrophages [28], but little is known about changes in COX-2 expression in the human brain during aging. Now that several inflammatory processes are thought to play a critical role in brain aging and to be associated with an increased vul-

nerability to neurodegeneration [29], we think that our finding provides a new point of view in regard to the aging brain. For example, a COX inhibitor was reported to protect mice from age-associated cognitive impairment under normal conditions [30]. These studies may illustrate a new 'anti-aging' treatment for the human brain.

In addition, we assessed neuropathologically confirmed AD patients in order to appreciate the influence of AD pathology on COX-2 expression within the hippocampus. We found that COX-2 immunoreactivity was increased in AD patients as compared to nondemented subjects and this change was observed in almost all hippocampal fields, reaching statistical significance in the CA1 field. This difference remained statistically significant even when we limited the nondemented subjects to those with AD pathology. In other words, the upregulation of COX-2 in the CA1 field in conjunction with AD pathology may be one of the important factors for developing AD. Over the last 10 years, several studies have examined the expression of COX-2 in postmortem AD brain tissues but have yielded conflicting results. One of the problems in these studies is in the method of selecting the nondemented group used for comparison with the AD group. As we mentioned, there is a possibility that COX-2 expression may be augmented with age; therefore, in a study of aged subjects, the difference of COX-2 expression between the AD and the nondemented group may become small and the opposite may also be true. Making the matter complicated, the degree of this augmentation may differ in each area.

We found that COX-2 immunoreactivity in CA1 correlates with AD pathology in AD patients. A previous immunohistochemical study reported a similar result [8], and recently the possibility of a direct interaction between human A β and COX-2 being mediated by peroxidase activity was reported [31]. Why the correlation between COX-2 immunoreactivity in the CA1 field and AD

pathology was observed only in AD patients and not in nondemented subjects is still unclear and further examination is required.

Here, we assessed the relationship between the neuronal expression of COX-2 and AD. It has recently been reported that the neuronal expression of COX-2 may play an important role in other types of neurodegenerative disorders. For instance, neuronal COX-2 expression in all CA fields of the hippocampus was significantly upregulated in ALS patients as compared to control subjects [13] and COX-2 was upregulated in brain dopaminergic neurons of both Parkinson's disease and MPTP-treated mice [12]. Upregulation of neuronal COX-2 expression may be a common pathway that a variety of neurodegenerative disorders exhibit.

In conclusion, in this study we have explored the regional expression of COX-2 in the hippocampus of the normal brain, and based on this result we have also explored the correlation between AD pathology and COX-2 expression in this region. While COX-2 expression appears to be differently regulated amongst hippocampal subdivisions and its presence may be augmented with age in the cognitively normal brain, COX-2 expression within the CA1 field of AD brains may be associated with the cognitive decline to some extent.

Acknowledgments

This study was supported in part by a Grant in Aid for the 21st Century COE program and Grant in Aid for Scientists (No. 16300112) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We are grateful to Dr. Takeshi Iwatsubo, Department of Neuropathology and Neuroscience, University of Tokyo, for generously donating the α -synuclein antibody LB509. We thank Mr. S. Mawatari and Ms. S. Nagae for their technical assistance.

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