(1)

学位論文

① Changes in the ratio of urinary α1-microglobulin to ulinastatin levels in patients with Alzheimer-type dementia and vascular dementia.
Psychiatry and Clinical Neuroscience 49, 287-290 (1995)

② Changes in the ratio of urinary α1-microglobulin to ulinastatin levels in patients with psychiatric diseases.
Biological Psychiatry (in press)

③ Non-existence of a positive correlation between urinary levels of α1-microglobulin and ulinastatin in patients with Parkinson's disease.
Psychiatry and Clinical Neuroscience (in press)

稲垣卓司

Psychiatry and Clinical Neurosciences (1995), **49**, 287–290

Regular Article

Changes in the ratio of urinary α1-microglobulin to ulinastatin levels in patients with Alzheimer-type dementia and vascular dementia

TAKUJI INAGAKI, MD,[1] TADAHIRO SHIKIMI, PHD,[2] HIROSHI ISHINO, MD,[1] HIDEKI OKUNISHI, MD[2] AND SHUJI TAKAORI, MD[2]

Department of [1]Psychiatry and [2]Pharmacology, Shimane Medical University, Izumo, Shimane, Japan

Abstract Relationships between urinary levels of α1-microglobulin (α1M) and ulinastatin (UT) in patients with dementia were investigated. There were no significant differences in α1M and UT levels and α1M : UT ratios among three groups: age-matched control subjects, patients with either Alzheimer-type senile dementia (ATD) or vascular dementia (VD). Although a positive correlation was established between α1M and UT levels in these groups, the regression of the demented patients differed significantly from that of controls ($P < 0.05$). A tendency towards a negative correlation between α1M : UT ratios and the levels of severity or duration of the disease was displayed in the ATD group, whereas a tendency toward a positive correlation between α1M : UT ratios and the levels of severity was observed in the VD group. These results suggest that changes in the relationships between urinary levels of α1M and UT may provide a useful biochemical index for diagnoses of ATD and VD.

Key words trypsin inhibitor, urine.

INTRODUCTION

A trypsin inhibitor found in human urine has been variously named Mingin,[1] H130[2] and Bikunin.[3] In Japan, ulinastatin (UT)* has been coined as the generic name for this substance, which is used for the treatment of acute pancreatitis. Both α1-microglobulin (α1M), a glycoprotein having an immunosuppressive activity,[4] and UT in human urine are derived from a common precursor protein in the liver.[5,6] However, the physiologically relevant functions of these substances have not been clarified as yet. The amino acid sequence of UT is highly homologous to the inhibitory domain of amyloid β-protein precursor.[7–9] Deposits of β-amyloid are one of the main pathological characteristics of Alzheimer's disease, and the importance of this domain in β-amyloid deposition in the brain has been demonstrated.[10] Moreover, a UT-like immunoreactive substance has been discovered in brain tissues of subjects with senile Alzheimer-type dementia.[11] In the rat and murine brain, a UT-like immunoreactive substance with trypsin inhibitory activities is found in sites related to memory and learning.[12,13] The glycoprotein, α1M, exists in various body fluids,[14] and variations in the levels of cerebrospinal fluid have been noted in certain neurological disorders.[15] These reports suggest that UT and α1M are related to psychoneuropathological conditions. The present study was carried out in order to investigate whether changes in urinary levels of UT and α1M were associated with dementia.

*The generic name of urinary trypsin inhibitor in English has been changed recently from urinastatin to ulinastatin, as the substance is found not only in urine but also in serum.

Correspondence address: Takuji Inagaki, MD, Department of Psychiatry, Shimane Medical University, Enya, Izumo, Shimane 693, Japan.

Received 6 March 1995; revised 5 June 1995; accepted 16 June 1995.

SUBJECTS AND METHODS

Fourteen patients with Alzheimer-type senile dementia (ATD) and 21 patients with vascular dementia (VD) were diagnosed according to DSM-III-R,[16] computed tomography of the brain, ischemic scores[17] and clinical symptoms.[18] Nondemented controls (NC; $n = 14$) without any history or symptoms of psychiatric and neurological diseases were included as reference cases. None of the subjects indicated hepatic and renal dysfunctions or other malignant diseases.

Severity of dementia

The severity of dementia was evaluated using the Hasegawa's dementia rating scale[19] accompanied by ranking their scores in activities of daily living.[20]

Assay of urine specimen

Urine spontaneously collected from the subjects was centrifuged at $1000 \times g$ for 10 min at 4°C in order to remove debris and amorphous salts prior to storage at −50°C until

assay. Alpha1M and UT levels were measured using the ELISA method, by interacting galactosidase labeled goat antirabbit IgG (Biotrin International, Doublin) with rabbit anti-α1M IgG (Dako Co., Copenhagen, Denmark) and rabbit anti-UT IgG,[21] respectively. Standard α1M (Dako Co.) and UT (a gift from Mochida Pharmaceutical Co., Tokyo, Japan) were used for reference. Creatinine contents in the urine were measured with a Wako Creatinine Kit (Wako Chemicals, Osaka, Japan) based on the Jaffe reaction.[22] Urinary levels of α1M and UT were expressed as μg/mg creatinine to correct for the dilution rate of urine samples.

Statistical analysis

Linear regression analyses were evaluated by using the STAT VIEW IV (Abacus Concept Inc., CA, USA), in which the least square method was used to obtain the slope and intercept of the regression line. Statistical significance was verified by unpaired *t*-test.

RESULTS

The age, α1M and UT levels and α1M : UT ratios did not differ significantly among the three groups (Table 1).

The existence of a significant correlation between UT and α1M concentrations in the urine was established (Fig. 1). The correlation coefficients in the NC, ATD and VD groups were 0.702 ($P < 0.01$), 0.886 ($P < 0.001$) and 0.871 ($P < 0.001$), respectively. The respective regression slopes in these dementia groups (1.65 in ATD, 1.26 in VD) were not statistically different. However, both regression slopes in the two dementia groups differed significantly ($P < 0.05$) from that of the NC group (slope 0.65).

Figure 2 illustrates the relationships between the α1M : UT ratios and the severity of dementia. A tendency toward negative correlations with low coefficients was revealed between the ratio and scores either from the dementia rating scale or the activities of daily living in the ATD group. In the VD group, however, a reversed tendency (positive correlations) was portrayed between the ratio and scores either from the dementia rating scale or the activities of daily living.

As the ATD group was composed exclusively of female patients the α1M/UT ratio of the scores of 13 female patients in the ATD group was compared with that of 14 female patients in the VD group. A pattern similar to that depicted in Fig. 2 was observed in the female cases, and the correlation coefficients between the α1M : UT ratio and dementia rating scale : activities of daily living in both the ATD and VD group were 0.225 : 0.324 and 0.525 : 0.489, respectively.

A correlation between the α1M: UT ratio and duration of the disease since the first symptoms in patients with severe dementia was manifested (Fig. 3). In the ATD group, a

Table 1. Sex, age, levels of α1-microglobulin (α1M) and ulinastatin (UT), and α1M/UT ratio in urine

	Nondemented control	Alzheimer-type dementia	Vascular dementia
No. subjects	14	14	21
Sex (male/female)	3/11	1/13	7/14
Age (years)	81 ± 2	78 ± 3	82 ± 2
α1M (μg/mg Cr)	11.53 ± 1.13	14.43 ± 4.22	12.05 ± 1.25
UT (μg/mg Cr)	7.85 ± 1.21	9.82 ± 2.27	8.06 ± 0.87
Ratio (α1M : UT)	1.67 ± 0.14	1.43 ± 0.15	1.56 ± 0.09

Values in age, levels of α1M and UT, and α1M : UT ratio are represented as mean ± s.e.m. Cr, creatinine in urine.

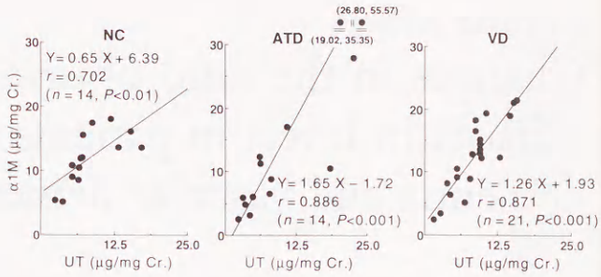


Figure 1. Correlation between urinary levels of ulinastatin (UT) and α1-microglobulin (α1M) in age-matched nondemented controls (NC) and patients with either Alzheimer-type sensile dementia (ATD) or vascular dementia (VD).

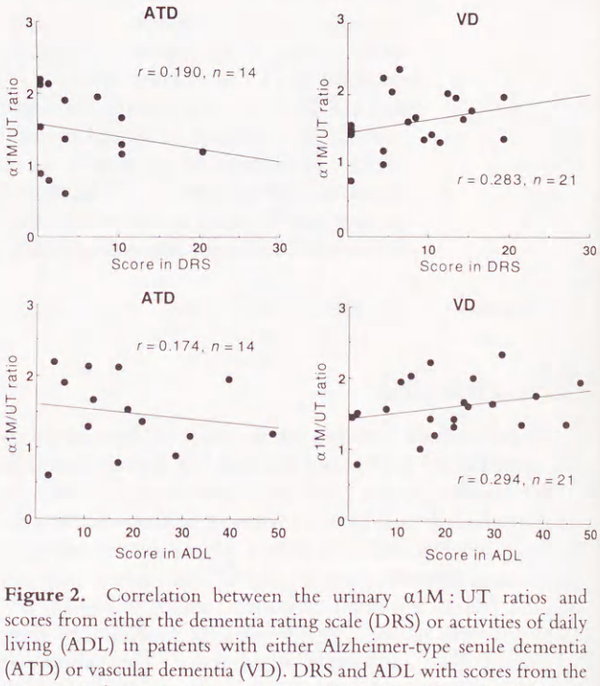


Figure 2. Correlation between the urinary α1M : UT ratios and scores from either the dementia rating scale (DRS) or activities of daily living (ADL) in patients with either Alzheimer-type senile dementia (ATD) or vascular dementia (VD). DRS and ADL with scores from the Haegawa's dementia rating scale and ADL of the elderly (N-ADL) are indicated, respectively.

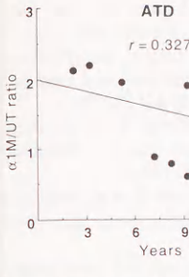


Figure 3. Correlatio
disease since the appea
Hasegawa's dementia
Alzheimer-type senile

longer duration of
in the ratio, wher
with the duration

DISCUSSION

Significant differe
and α1M : UT rati
and VD groups. T
α1M and UT and
index for the diag
sion slope in the
indicated a signific
than in the age-ma
suggest that the
α1M levels differ
Although the regr
between the ATD
noted in the relati
dementia and dura
relation of urina
meaningful than
UT and α1M : U
other words, char
useful biochemica
ment of dementia.

Certain cytokin
may be involved i
death and amyloid
cytokine receptors
kines associated
been reported. In
which α1M and U
elements[27] and ir
found. In addition
UT in urine have
mechanisms of
changes in neuroi
system and/or in
in dementia patie
tionship between

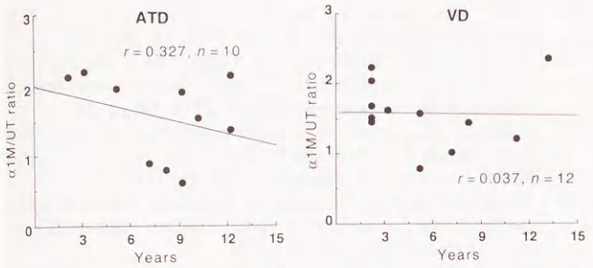


Figure 3. Correlation between α1M : UT ratios and duration of the disease since the appearance of the first symptoms. The scores from the Hasegawa's dementia rating scale were under 10 in patients with either Alzheimer-type senile dementia (ATD) or vascular dementia (VD).

longer duration of the disease corresponded to a lower value in the ratio, whereas the α1M : UT ratio was not altered with the duration in VD patients.

DISCUSSION

Significant differences in the urinary levels of α1M and UT and α1M : UT ratio were not detected among the NC, ATD and VD groups. These results suggest that urinary levels of α1M and UT and α1M : UT ratio *per se* cannot serve as an index for the diagnosis of dementia. However, the regression slope in the α1M : UT (concentration) relationship indicated a significantly higher level in the dementia groups than in the age-matched controls ($P < 0.05$). These findings suggest that the relationships between excreted UT and α1M levels differ between the dementia and NC groups. Although the regression slope was not statistically different between the ATD and VD groups, different patterns were noted in the relation of α1M : UT ratios to the severity of dementia and duration of the disease. As such, changes in the relation of urinary levels of α1M and UT were more meaningful than measuring the urinary levels of α1M and UT and α1M : UT ratio for the diagnosis of dementia. In other words, changes in the α1M : UT ratio may provide useful biochemical information on the type and development of dementia.

Certain cytokines regulating glucocorticoid production[23] may be involved in dystrophic neuritic sprouting, neuronal death and amyloid deposition.[24] In the brain, the presence of cytokine receptors[25] and of differential production of cytokines associated with different types of dementia[26] have been reported. In the gene of the precursor protein from which α1M and UT are derived, glucocorticoid responsive elements[27] and interleukin 6 sequence motifs[3] have been found. In addition, glucocorticoid-associated fluctuations of UT in urine have been observed.[28,29] Although the exact mechanisms of present findings warrant further study, changes in neuroimmune mechanisms in the central nervous system and/or in cytokine concentrations at peripheral sites in dementia patients may be involved in the variable relationship between urinary levels of α1M and UT.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan. The authors would like to thank Drs T. Sakurai (Konan Hospital, Shimane) and H. Kanno (Matsue Aoba Hospital, Shimane) and colleagues in the Department of Psychiatry, Shimane Medical University for their support in this study.

REFERENCES

1. Faarvang HJ. Urinary trypsin inhibitor in man ("mingin"). *Scand. J. Clin. Lab. Invest.* 1965; **17** (Suppl. 83): 1–78.
2. Wachter E, Hochstrasser K. Kunitz-type protease inhibitors derived by limited proteolysis of the inter-α-trypsin inhibitor, IV: The amino acid sequence of the human urinary trypsin inhibitor isolated by affinity chromatography. *Hoppe-Seyer's J. Physiol. Chem.* 1981; **362**: 1351–1355.
3. Vetr H, Gebhard W. Structure of the human α1-microglobulin-bikunin gene. *Biol. Chem. Hoppe-Seyler* 1990; **371**: 1185–1196.
4. Åkerström B, Lögdberg L. An intriguing member of the lipocalin protein family: α_1-microglobulin. *Trends Biochem. Sci.* 1990; **15**: 240–243.
5. Kaumeyer JF, Polazzi JO, Kotick MP. The mRNA for a protinease inhibitor related to the HI-30 domain of inter-α-trypsin inhibitor also encodes α-1-microglobulin (protein HC). *Nucleic Acids Res.* 1986; **14**: 7839–7850.
6. Schreitmüller T, Hochstrasser K, Reisinger PWM *et al.* cDNA cloning of human inter-α-trypsin inhibitor discloses three different proteins. *Biol. Chem. Hoppe-Seyler* 1987; **368**: 963–970.
7. Kitaguti N, Takahashi Y, Tokushima Y *et al.* Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. *Nature* 1988; **331**: 530–532.
8. Ponte P, Gonzalez-Dewhitt P, Schilling J *et al.* A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. *Nature* 1988; **331**: 525–527.
9. Tanzi RE, McClatchy AI, Lamperti ED *et al.* Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* 1988; **331**: 528–530.
10. Quon D, Wang Y, Catalano R *et al.* Formation of β-amyloid protein deposits in brains of transgenic mice. *Nature* 1991; **352**: 239–241.
11. Yoshida E, Yoshimura M, Itoh Y, Mihara H. Demonstration of an active component of inter-α-trypsin inhibitor in the brains of Alzheimer type dementia. *Biochem. Biophys. Res. Comm.* 1991; **174**: 1015–1021.
12. Shikimi T, Hattori K, Takaori S. Existence of a human urinary trypsin inhibitor (urinastatin)-like substance in the rat brain. *Jpn. J. Pharmacol.* 1992; **60**: 97–103.
13. Shikimi T, Wessel T, Joh TH *et al.* Demonstration of a human urinary trypsin inhibitor (urinastatin)-like substance in the murine brain. *Brain Res.* 1993; **616**: 230–235.
14. Takagi K, Kin K, Itoh Y *et al.* Human α_1-microglobulin levels in various body fluids. *J. Clin. Pathol.* 1980; **33**: 786–791.
15. Itoh Y, Enomoto H, Takagi K *et al.* Human alpha 1-microglobulin levels in neurological disorders. *Eur. Neurol.* 1983; **22**: 1–6.
16. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 3rd edn revised. American Psychiatric Association, Washington DC, 1987.
17. Hachinski VC, Iliff LD, Zilhka E *et al.* Cerebral blood flow in dementia. *Arch Neurol.* 1975; **32**: 632–637.
18. McKeith I. The differential diagnosis of dementia. **In**: Burns A, Levy R (eds.) *Dementia*. Chapman and Hall Medical, New York, 1992; 39–57.

19. Katoh S, Simogaki H, Onodera A *et al.* Development of the revised version of Haegawa's dementia scale (HDR-S). *Geronto-psychiatry (Ronen Seishinigaku Zashi)* 1991; **2**: 1339–1347 (in Japanese).
20. Kobayashi T, Hataguchi T, Nishimura K *et al.* A new clinical scale for rating of mental states and activities of daily living of the elderly (NM scale and N-ADL). *Jpn. J. Clin. Psych. (Rinsho Seishinigaku)* 1988; **17**: 1653–1668. (in Japanese).
21. Shikimi T, Suzuki S, Takahashi M, Kaneto H. Sandwich enzyme-immunoassay of human urinary trypsin inhibitor (urinastatin) and urinastatin-like immunoreactive substance in mouse urine. *Scand. J. Clin. Lab. Invest.* 1990; **50**: 1–8.
22. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* 1945; **158**: 581–591.
23. Sapolsky R, River C, Yamamoto G *et al.* Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 1987; **238**: 522–524.
24. Altstiel LD, Sperber K. Cytokines in Alzheimer's disease. *Prog. Neuro Psychopharmacol. Biol. Psychiat.* 1991; **15**: 481–495.
25. Ban E, Milon G, Prudhomme N, Fillion G *et al.* Receptors for interleukin-1 (α and β) in mouse brain: Mapping and neuronal localization in hippocampus. *Neurosci.* 1991; **43**: 21–30.
26. Cacabelos R, Alvarez XA, Fernandez-Novoa L *et al.* Brain interleukin-1 β in Alzheimer's disease and vascular dementia. *Method Find Exp. Clin. Pharmacol.* 1994; **16**: 141–151.
27. Diarra-Mehrpour M, Bourguignon J, Sesboüé R *et al.* Structural analysis of the human inter-α-trypsin inhibitor light-chain gene. *Eur. J. Biochem.* 1990; **191**: 131–139.
28. Faarvang HJ. The influence of glucocorticoids and corticotropic hormone on output of human urinary trypsin inhibitor (and hyaluronidase inhibitor). *Acta Pharmacol. Toxicol.* 1962; **19**: 293–304.
29. Shikimi T, Suzuki S, Wessel T *et al.* Human urinary trypsin inhibitor (urinastatin)-like substance in mouse liver. *Life Sci.* 1992; **50**: 1399–1406.

Changes in the Ratio of Urinary α1-Microglobulin to Ulinastatin Levels in Patients with Psychiatric Diseases

Takuji Inagaki, Tadahiro Shikimi, Akihiko Fujimoto, Hiroshi Ishino, Hideki Okunishi, and Shuji Takaori

Department of Psychiatry (TI, AF, HI) and Department of Pharmacology (TS, HO, ST), Shimane Medical University, Izumo, Shimane 693, Japan

Introduction

Alpha-1-Microglobulin (α1M, 31kDa) and ulinastatin* (UT, 40kDa) in human urine are derived from a similar precursor protein in the liver (Kaumeyer et al 1986). Hitherto, the physiologically relevant functions of these substances have not been clarified. The amino acid sequence of UT is not only highly homologous to the inhibitory domain of amyloid β-protein precursor associated with senile plaque or amyloid angiopathy (Kitaguti et al 1988; Ponte et al 1988; Tanzi et al 1988), but a UT-like immunoreactive substance with trypsin inhibitory activities is also found in brain sites related to memory and learning (Shikimi et al 1993). The relationships of urinary α1M and UT levels have been previously found to differ between the dementia and aged-matched control groups. In addition, different patterns of α1M/UT ratio in relation to the severity of dementia and duration of the disease have been displayed between Alzheimer-type senile dementia and vascular dementia patients (Inagaki et al 1995). In the present study, we studied further whether or not the relation between urinary levels of α1M and UT differed in patients with psychoses.

---

* The generic name of this human urinary trypsin inhibitor has been recently changed from urinastatin to ulinastatin, as the substance is found not only in urine but also in serum.

Methods

Twelve patients with schizophrenia and 19 patients with mood disorder (10, patients with bipolar affective disorder; 9, patients with major depression) were diagnosed according to the DSM-Ⅲ-R (American Psychiatric Association 1987). Patients over 60 years of age were excluded in this study. The durations of schizophrenia and mood disorder in patients were 10.4 ± 2.3 and 6.1 ± 1.8 years (mean ± S.E.M), respectively. The respective severities of schizophrenia and mood disorder patients were 43 ± 6 (Brief Psychiatry Rating Scale, Overall and Gorham 1962) and 24 ± 4 points (Hamilton Depression Rating Scale, Hamilton 1960)(mean ±S.E.M). Patients with bipolar affective disorder (6 patients with and 4 without lithium treatment for at least 4 months) indicated neither manic nor depressive episodes for at least 30 days. All the patients with major depression were treated with antidepressants. As age-matched controls, 18 subjects without any present or past history of psychiatric diseases were included. None of the subjects indicated hepatic/renal dysfunctions or other malignant diseases. Informed consent was obtained from all subjects before the study.

Spontaneously collected urine (from the subjects) was centrifuged at 1,000 x g for 10 min at 4°C to remove debris and amorphous salts prior to storage at -50°C until assay. Urinary levels of α1M and UT were measured with ELISA, whereby galactosidase-labelled goat anti-rabbit IgG was interacted

(Biotrin International, Dublin) with rabbit anti-α1M IgG (Dako Co., Copenhagen) and rabbit anti-UT IgG (Shikimi et al 1990), respectively. Standard α1M (Dako Co., Copenhagen) and UT were used as references. Creatinine levels in the urine were measured with a photometric assay based on the Jaffe reaction (Wako creatinine kit, Wako Chemicals, Osaka). Urinary levels of α1M and UT were expressed as μg/mg creatinine to correct for the dilution rate of urine samples.

Linear regression analyses were evaluated by using the Stat View IV system (Abacus Concept, Inc., CA). Statistical significance was verified by the factorial ANOVA followed by Scheffe's post hoc test. Values where $p<0.05$ were considered statistically significant.

Results

In the bipolar affective disorder group, significant differences in the age and urinary levels of creatinine, α1M and UT, and α1M/UT ratio were not detected between the lithium-treated and untreated patients. The lithium-treated and untreated patients were thus pooled as a bipolar affective disorder group. In the Table 1, age and levels of creatinine, α1M and UT did not differ

significantly among the control, schizophrenia, mood disorder groups and its subdivided groups (bipolar affective disorder and major depression). The α1M/UT ratio of the mood disorder group, but not the schizophrenia group, was significantly higher than that of the controls ($P<0.01$). The bipolar affective disorder patients, but not the major depression cases, contributed to this change.

Relating urinary levels of UT and α1M, a positive correlation was observed in the control, schizophrenia and mood disorder groups, accordingly (Fig. 1). A similar tendency was manifested by the bipolar affective disorder and major depression groups (Fig. 2). Although the regression slope of the schizophrenia cases was not statistically different from that of controls, a significant difference was noted between the mood disorder and control groups ($P<0.05$). Between the schizophrenia and mood disorder groups, the regression slope was significantly ($P<0.05$) different. Furthermore, the regression slope of the major depression group, but not the bipolar affective disorder group, was statistically ($P<0.05$) different from those of controls and schizophrenia groups. In the schizophrenia and mood disorder groups, neither the scores from psychiatric rating scales (Brief Psychiatric Rating Scale and Hamilton Depression Rating Scale) nor durations of disease correlated well with the urinary levels of α1M or UT, or α1M/UT ratio.

Discussion

Comparing the schizophrenia and control groups, there were no significant differences in the regression slopes, urinary levels of α1M and UT, and α1M/UT ratio. Furthermore, neither the scores of Brief Psychiatric Rating Scale nor durations of the diseases correlated well with the urinary levels of α1M or UT. These findings suggest that the urinary levels of α1M and UT and α1M/UT ratio are not influenced by the pathophysiology of schizophrenia.

Comparing the mood disorder and control groups, the regression slope of the former differed from that of controls. Within the mood disorder category, the slope of the major depression group was especially significant ($P<0.05$). Furthermore, the α1M/UT ratio of mood disorder was significantly ($P<0.01$) higher than that of controls. Within the mood disorder category, the α1M/UT ratio of the bipolar affective disorder group was particularly significant ($P<0.01$). These results suggest that the relationships between the urinary levels of α1M and UT in mood disorder as a whole differ from those of controls.

Comparing the mood disorder category (and inclusive of bipolar affective disorder and major depression) with schizophrenia patients, the regression slopes of the mood disorder as a whole

and major depression groups differed from that of schizophrenia.

In our previous study (Inagaki et al 1995), a positive correlation between urinary levels of α1M and UT has been observed in dementia patients. The respective regression slopes of dementia groups (Alzheimer-type and vascular-type) were significantly different from that of non-demented controls in spite of insignificant difference in the α1M/UT ratio. Therefore, the relation between urinary levels of α1M and UT differed among the schizophrenia, mood disorder as a whole and dementia patients.

The α1M/UT ratio of bipolar affective disorder group was significantly higher than that of controls. However, lithium medication (60 % in the group were medicated with lithium) did not affect the change. This was advocated by insignificant differences in the urinary levels of creatinine, α1M and UT and α1M/UT ratio between lithium-treated and untreated patients. With regard to diet, variations in the urinary levels of α1M and UT and α1M/UT ratio were not observed. In addition, even in patients with urological diseases, the regression slope relating to urinary levels of α1M and UT remained statistically insignificant from that of healthy subjects (Shikimi et al 1994). However, the regression slope of the mood disorder group differed from that of controls. As none of the present subjects indicated

any renal dysfunctions, renal factors contributing to changes in the relation between urinary levels of α1M and UT in mood disorder patients may be discounted.

Glucocorticoid-responsive elements (Diarra-Mehrpour et al 1990) and interleukin 6 sequence motifs (Vetr and Gebhard 1990) have been found in a gene of the α1M- and UT-derived precursor protein. In addition, glucocorticoid-associated fluctuations of UT in urine, in which plasma levels of glucocorticoids are reportedly affected by cytokines (Spolsky et al 1987; Hermus and Sweep 1990), have been observed (Faarvang 1962). The urinary levels of α1M, UT and α1M/UT ratio are also influenced by the forms (free and complexed) of α1M and UT existing in plasma (α1M and UT complexes respectively exist in IgA-complexed (Demars et al 1989) and IgG-complexed forms (Hochstrasser et al 1981)), since only the free forms of these substances are found in urine (Jönsson-Berling et al 1989; Demars et al 1989). In the etiology of psychosocial diseases, the immune system has been focused as a key factor (Smith 1991). Changes in the systemic immune system, neuroimmune mechanisms in the central nervous system and/or cytokine concentrations at peripheral sites in psychoses may be associated with the variable relationships between urinary levels of α1M and UT. Although the exact mechanism(s) and physiological significance of the α1M/UT ratio warrant further studies, our

present findings suggest that a change in the relationship between urinary levels of α1M and UT reflects the psychoneurological differences underlying the various psychiatric diseases in human.

Acknowledgements

The authors thank Dr. T. Sakurai (Konan Hospital, Shimane) and colleagues in the Department of Psychiatry, Shimane Medical University for providing the urine samples of subjects for this study. Standard UT was a kind gift from Mochida Pharmaceutical Co., Tokyo.

References

American Psychiatric Association (1987): Diagnostic and Statistical Manual of Mental Disorders, 3rd ed, rev. Washington DC: American Psychiatric Association.

Demars DD, Katzmann JA, Kimlinger TK, Calore JD, Tracy RP (1989): Simultaneous measurement of total and IgA-conjugated α1-microglobulin by a combined immunoenzyme/immunoradiometric assay technique. Clin Chem 35:766-772.

Diarra-Mehrpour M, Bourguignon J, Sesboüé R, Salier J-P, Léveillard T, Martin J-P (1990): Structural analysis of the human inter-α-trypsin inhibitor light-chain gene. Eur J Biochem 191:131-139.

Faarvang HJ (1962): The influence of glucocorticoids and corticotropic hormone on output of human urinary trypsin inhibitor (and hyaluronidase inhibitor). Acta Pharmacol Toxicol 19:293-304.

Hamilton M (1960): A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56-61.

Hermus AR, Sweep CG (1990): Cytokines and the hypothalamic-pituitary-adrenal axis. J Steroid Biochem Mol Biol 37:867-871.

Hochstrasser K, Schönberger ÖL, Lempart K, Metzger M (1981): Kunitz-type proteinase inhibitors derived by limited

proteolysis of the inter-α-trypsin inhibitor. VI. Detection of a complex between immunoglobulin G and the inhibitory active part of the inter-α-trypsin inhibitor. Hoppe-Seyler's Z Physiol Chem 362:1363-1367.

Inagaki T, Shikimi T, Ishino H, Okunishi H, Takaori S (1995): Changes in the ratio of urinary α1-microglobulin to ulinastatin levels in patients with Alzheimer-type and vascular dementia. Psychiary Clin Neurosci 49:287-290.

Jönsson-Berling B-M, Ohlsson K, Rosengren M (1989): Radioimmunological quantitation of the urinary trypsin inhibitor in normal blood and urine. Biol Chem Hoppe-Seyler 370:1157-1161.

Kaumeyer JF, Polazzi J, Kotick MP (1986): The mRNA for a protease inhibitor related to the HI-30 domain of inter-α-trypsin inhibitor also encodes α-1-microglobulin (protein HC). Nucleic Acids Res 14:7839-7850.

Kitaguti N, Takahashi Y, Tokushima Y, Shiojiri S, Ito N (1988): Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. Nature (London) 331:530-532.

Overall JE, Gorham DR (1962): The brief psychiatric rating scale. Psychol Rep 10:799-812.

Ponte P, Gonzalez-Dewhitt P, Schilling J, Miller J, Hsu D, Greenberg B, Davis K, Wallace W, Lieberburg I, Fuller F,

Cordell B (1988): A new A4 amyloid mRNA contains a domain homologus to serine proteinase inhibitors. Nature (London) 331:525-527.

Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W (1987): Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238:522-524 (1987)

Shikimi T, Suzuki S, Takahashi M, Kaneto H (1990): Sandwich enzyme-immunoassay of human urinary trypsin inhibitor (urinastatin) and urinastatin-like immunoreactive substance in mouse urine. Scand J Clin Lab Invest 50:1- 8.

Shikimi T, Wessel T, Joh TH, Takahashi M, Kaneto H, Hattori K, Takaori S (1993): Demonstration of a human urinary trypsin inhibitor (urinastatin)-like substance in the murine brain. Brain Res 616:230-235 .

Shikimi T, Himeno Y, Shigeno K, Gonda T, Ishibe T, Hattori K, Takaori S (1994): Relationships between ulinastatin and alpha-1-microglobulin in human urine. Clin Chim Acta 227: 195-200.

Smith RS (1991): The immune system is a key factor in the etiology of psychosocial disease. Med Hypoth 34:49-57.

Tanzi RE, McClatchey AI, Lamperti ED, Villa-Komaroff L, Gusella JF, Neve RL (1988): Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature (London) 331:528-530.

Vetr H, Gebhard W (1990): Structure of the human α1-micro-globulin-bikunin gene. Biol Chem Hoppe-Seyler 371:1185-1196.

Table 1. Number, sex, age, levels of creatinine, α1-microglobulin (α1M) and ulinastatin (UT), and α1M/UT ratio in urine of subjects

	Normal Control	Schizophrenia	Mood disorder		
			Bipolar	Depression	Total
Number of Subjects	18	12	10	9	19
Sex (M/F)	10/8	6/6	5/5	5/4	10/9
Age (years)	40 ± 2	34 ± 3	42 ± 3	33 ± 4	38 ± 3
Cr (mg/ml)	1.37 ± 0.16	0.97 ± 0.24	0.96 ± 0.17	1.37 ± 0.12	1.15 ± 0.11
α1M (μg/mg Cr)	6.87 ± 0.81	6.56 ± 0.83	9.77 ± 1.13	6.90 ± 1.21	8.41 ± 0.87
UT (μg/mg Cr)	10.34 ± 1.33	7.75 ± 1.39	6.76 ± 1.20	5.22 ± 0.77	6.03 ± 0.73
Ratio (α1M/UT)	0.75 ± 0.09	1.16 ± 0.21	1.65 ± 0.17[a)]	1.36 ± 0.15	1.51 ± 0.12[a)]

Values in age and urinary levels of creatinine (Cr), α1M, UT and α1M/UT ratio are represented as mean ± S.E.M.. Abbreviations: Bipolar, bipolar affective disorder; Depression, major depression. Superscript a) represents values (where $P<0.01$) significantly different from those of controls.

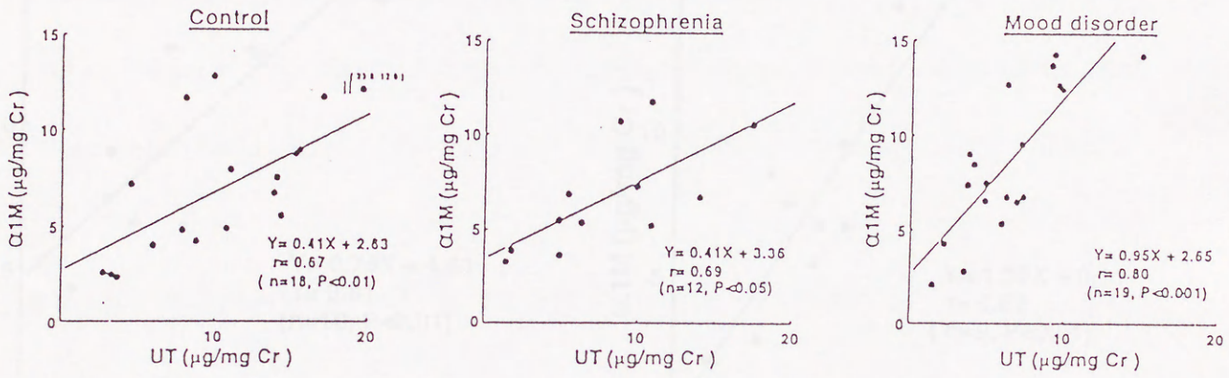


Fig.1. Correlation between urinary levels of ulinastatin (UT) and α1-microglobulin (α1M) in age-matched controls and patients with either schizophrenia or mood disorders. The correlation coefficient and number of subjects are represented with r and n in parentheses, respectively. Cr represents creatinine in urine.

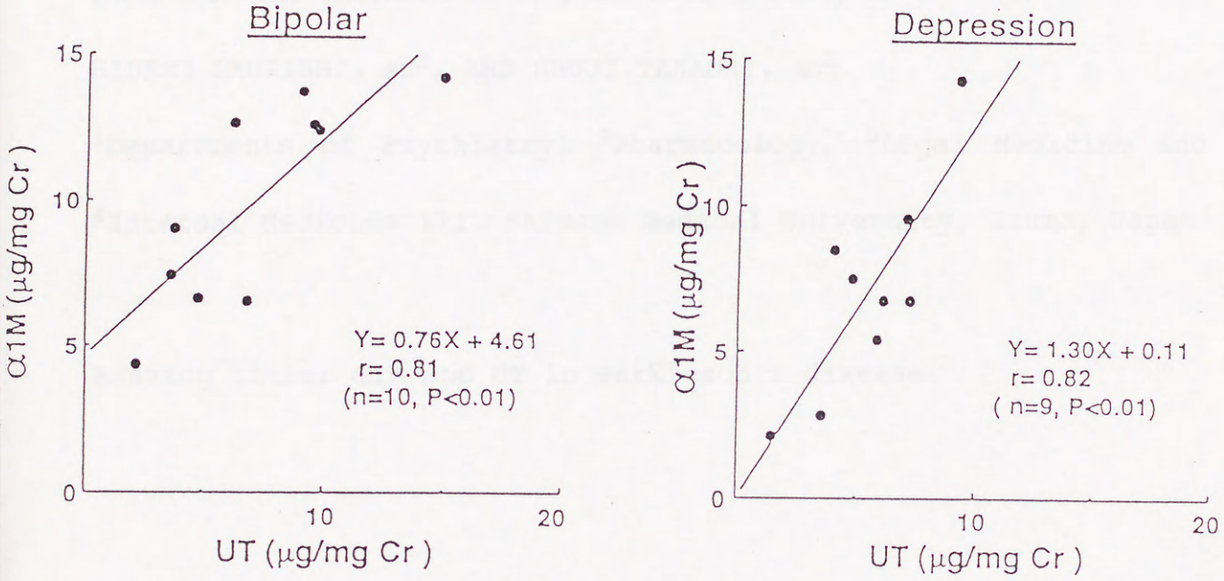


Fig.2. Correlation between urinary levels of ulinastatin (UT) and α1-microglobulin (α1M) in patients with bipolar affective disorder (Bipolar) or major depression (Depression). The correlation coefficient and number of subjects are represented with r and n in parentheses, respectively. Cr represents creatinine in urine.

Nonexistence of a positive correlation between urinary levels of α1-microglobulin and ulinastatin in patients with Parkinson's disease

TAKUJI INAGAKI, MD[1], TADAHIRO SHIKIMI, PHD[2], KAZUO MATSUBARA, PHD[3], SHOUTAI KOBAYASHI, MD[4], HIROSHI ISHINO, MD[1], HIDEKI OKUNISHI, MD[2], AND SHUJI TAKAORI, MD[2]

[1]Departments of Psychiatry, [2]Pharmacology, [3]Legal Medicine and [4]Internal Medicine III, Shimane Medical University, Izumo, Japan

Running title: α1M and UT in Parkinson's disease

Correspondence address: Takuji Inagaki, MD, Department of Psychiatry, Shimane Medical University, Enya, Izumo, Shimane 693, Japan.

Tel:(0853)23-2111 (Ext.2475)

Fax:(0853)21-9680

Abstract

Urinary levels of α1-microglobulin (α1M) and ulinastatin (UT) and α1M/UT ratio did not differ significantly (i) between age-matched control and Parkinson's disease groups and (ii) among subdivided groups based on Yahr's stages in Parkinson's disease. Further, these indexes did not correlate well with Yahr's stages. Although α1M and UT levels did not correlate in patients with Parkinson's disease, a positive correlation was observed in the control group. The nonexistence of a positive correlation between α1M and UT levels distinguishes Parkinson's disease from other neuropsychiatric diseases such as dementia (Alzheimer-type and vascular dementia), schizophrenia and mood disorder.

INTRODUCTION

Alpha-1-microglobulin (α1M) and ulinastatin* (UT) are found in human urine. Although these two substances are derived from a common precursor protein in the liver,[1] they are structurally unrelated glycoproteins. While α1M displays immunosuppresive activities[1] and UT elicits trypsin inhibitory activities[2], their physiologically relevant functions are still obscure. We have previously found that the relationship between urinary α1M and UT levels of dementia group differed from that of the age-matched non-demented control, and in the relation of α1M/UT ratios to the severity of dementia and duration of the disease, different patterns were noted between the Alzheimer-type senile dementia and vascular dementia.[3] In the present study, the relationship between urinary α1M and UT levels in patients with Parkinson's disease were investigated.

MATERIALS AND METHODS

Sampling and storage

Urine was spontaneously collected from healthy subjects without

---

* The generic name of urinary trypsin inhibitor has been recently changed from urinastatin to ulinastatin, as the substance is found in not only urine but also serum.

any history or symptoms of psychiatric and neurological diseases and in- or out-patients suffering from Parkinson's disease in Shimane Medical University. Samples were centrifuged at 1,000 x g for 10 min at 4°C prior to storage at -50°C until assay. The severity of Parkinson's disease was evaluated according to Yahr's stages,[4] and none of the subjects indicated hepatic and renal dysfunctions or other malignant diseases. Informed consent was obtained from all subjects after the purpose of the present study was explained to them.

Determination of α1M, UT and creatinine contents

Urinary α1M and UT levels were measured (ELISA method) by interacting galactosidase-labelled goat antirabbit IgG (Biotrin International, Doublin) with rabbit anti-α1M IgG (Dako Co., Copenhagen) and rabbit anti-UT IgG,[5] respectively. Standard α1M (Dako Co., Copenhagen) and UT (a kind gift from Mochida Pharmaceutical Co., Tokyo) were used as references. Creatinine contents in the urine were measured with a Wako Creatinine Kit (Wako Chemicals, Osaka). Urinary α1M and UT contents were expressed as μg/mg creatinine to correct for the dilution rate of urine samples.

Statistical analysis

Significant differences of α1M and UT contents and α1M/UT ratios between healthy subjects and patients with Parkinson's disease and among the three subdivided groups based on Yahr's stages were verified with the unpaired t-test and factorial ANOVA followed by Scheffe's post hoc test, respectively. Linear regression analyses and Spearman's rank correlation between the stages in patients with Parkinson's disease and respective α1M and UT levels and α1M/UT ratios were evaluated by using the Stat View IV (Abacus Concept, Inc., CA). P values of less than 0.05 were considered significant.

RESULTS

The α1M and UT levels and α1M/UT ratio between the control and Parkinson's disease groups and among the three subdivided groups (based on Yahr's stages) did not differ significantly (Table 1). With regard to subjects (composed exclusively of female patients) in both the control and Parkinson's disease groups, the α1M, UT levels and α1M/UT ratio did not display any statistical significances. In the patients with Parkinson's disease, significant correlations between the stages and respective α1M and UT levels and α1M/UT ratios were not observed when the Spearman's rank correlation was employed.

The existence of a positive correlation between the UT and α1M levels in urine was significantly observed in the control but not Parkinson's disease group (Figure 1). Female subjects both in the control and Parkinson's disease groups showed a pattern similar to that depicted in Figure 1. The correlation coefficients between the UT and α1M contents in the control and Parkinson's disease groups were 0.939 (p value in the regression, $P<0.002$; $n=7$) and 0.195 (p value in the regression, $P=0.505$; $n=14$), respectively.

DISCUSSION

Significant differences in the urinary α1M and UT levels and α1M/UT ratios were not detected between the control and Parkinson's disease groups. A similar tendency was indicated among the subdivided groups of patients with Parkinson's disease, though the cases of each stage were small in number. Furthermore, there was no significant correlation between the severity of Parkinson's disease and the respective levels of α1M and UT and α1M/UT ratios. These findings suggest that urinary levels of α1M and UT and the α1M/UT ratio per se cannot serve as useful indexes for the diagnosis and monitoring of the development of Parkinson's disease.

Contrary to controls, demented patients (both Alzheimer-type dementia and vascular dementia),[3] patients with schizophrenia and mood disorder (both bipolar affective disorder and major depression) (unpublished data), a positive correlation between urinary levels of α1M and UT was not observed in the patients with Parkinson's disease. Regarding nonexistence of the positive correlation, renal factors might not be a factor, since positive correlation is observed even in patients with urological diseases,[6] and none of the present subjects indicated any renal dysfunctions. In our investigation with C57 BL/6J mice, an animal species reportedly possesses the gene similar to that of humans for synthesizing the precursor protein of α1M and UT,[7] L-dopa treatment (50 mg/kg, i.v.; 25 mg/kg/day, s.c. for 7 consecutive days) did not affect the positive correlation in non-treated mice, whereas repetitive MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces pathological changes similar to human idiopathic Parkinson's disease in animals[8]) administrations (20 mg/kg, i.p., 4 injections every 12 hours) resulted in nullifying the positive correlation (unpublished data). These results suggest that the nonexistence of the correlation is closely associated with the neuropathological changes in Parkinson's disease, but not with L-DOPA treatment, though all the patients with Parkinson's disease were medicated

with L-DOPA. Although the exact mechanism warrants further studies, our present findings revealed an interesting feature between urinary levels of α1M and UT in Parkinson's disease.

ACKNOWLEDGEMENTS

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Åkerström B, Lögdberg L. An intriguing member of the lipocalin protein family: α_1-microglobulin. Trends Biochem. Sci. 1990; **15:** 240-243.
2. Shikimi T, Hattori K, Takaori S. Effects of heparin on the inhibitory activities of human urinary trypsin inhibitor (ulinastatin) on trypsin, chymotrypsin and leukocyte elastase. Jpn. J. Pharmacol. 1993; **62:** 115-118.
3. Inagaki T, Shikimi T, Ishino H, Okunishi H, Takaori S. Changes in the ratio of urinary α1-microglobulin to ulinastatin levels in patients with Alzheimer-type dementia and vascular dementia. Psychiatry Clin. Neurosci. 1995; **49:** 287-290
4. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967; **17:** 427-442.
5. Shikimi T, Suzuki S, Takahashi M, Kaneto H. Sandwich enzyme-immunoassay of human urinary trypsin inhibitor (urinastatin) and urinastatin-like immunoreactive substance in mouse urine. Scand. J. Clin. Lab. Invest. 1990; **50:** 1-8.
6. Shikimi T, Himeno Y, Shigeno K et al. Relationships between ulinastatin and alpha-1-microglobulin in human urine. Clin. Chim. Acta. 1994; **227:** 195-200.
7. Salier JP, Verga V, Doly J, Diarra-Mehrpour M, Erickson

RP. The genes for the inter-α-inhibitor family share a homologous organization in human and mouse. Mammal Genome 1992; **2:** 233-239.

8. Arai N, Misugi K, Goshima Y, Misu Y. Evaluation of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated C57 black mouse model for parkinsonism. Brain Res. 1990; **515:** 57-63.

Table 1. Number, sex, age, levels of α1-microglobulin (α1M) and ulinastatin (UT), and α1M/UT ratios in urine of normal and patients with Parkinson's disease

	Normal control	Parkinson's disease			
		stage 2	stage 3	stage 4	Total
Number of subjects	11	3	7	5	15
Sex (M/F)	4 / 7	1 / 2	0 / 7	0 / 5	1 / 14
Age (years)	63 ± 2 (64 ± 1)	50 ± 10	63 ± 3	62 ± 7	60 ± 3 (61 ± 3)
α1M (μg/mg Cr.)	11.01 ± 2.15 (12.41 ± 3.09)	5.01 ± 2.38	8.49 ± 2.28	5.66 ± 2.20	6.85 ± 1.28 (6.81 ± 1.38)
UT (μg/mg Cr.)	8.12 ± 1.57 (7.66 ± 1.91)	5.35 ± 3.94	6.77 ± 1.12	8.25 ± 2.34	6.98 ± 1.04 (6.66 ± 1.06)
Ratio (α1M/UT)	1.51 ± 0.21 (1.71 ± 0.23)	1.53 ± 0.62	1.47 ± 0.49	0.76 ± 0.25	1.25 ± 0.26 (1.29 ± 0.27)

Patients with Parkinson's disease were subdivided based on Yahr's stages. Values in age and urinary levels of α1M and UT and α1M/UT ratio are represented as mean ± S.E.M.. Values in parentheses are those of female cases. M, male; F, female; Cr., creatinine in urine.

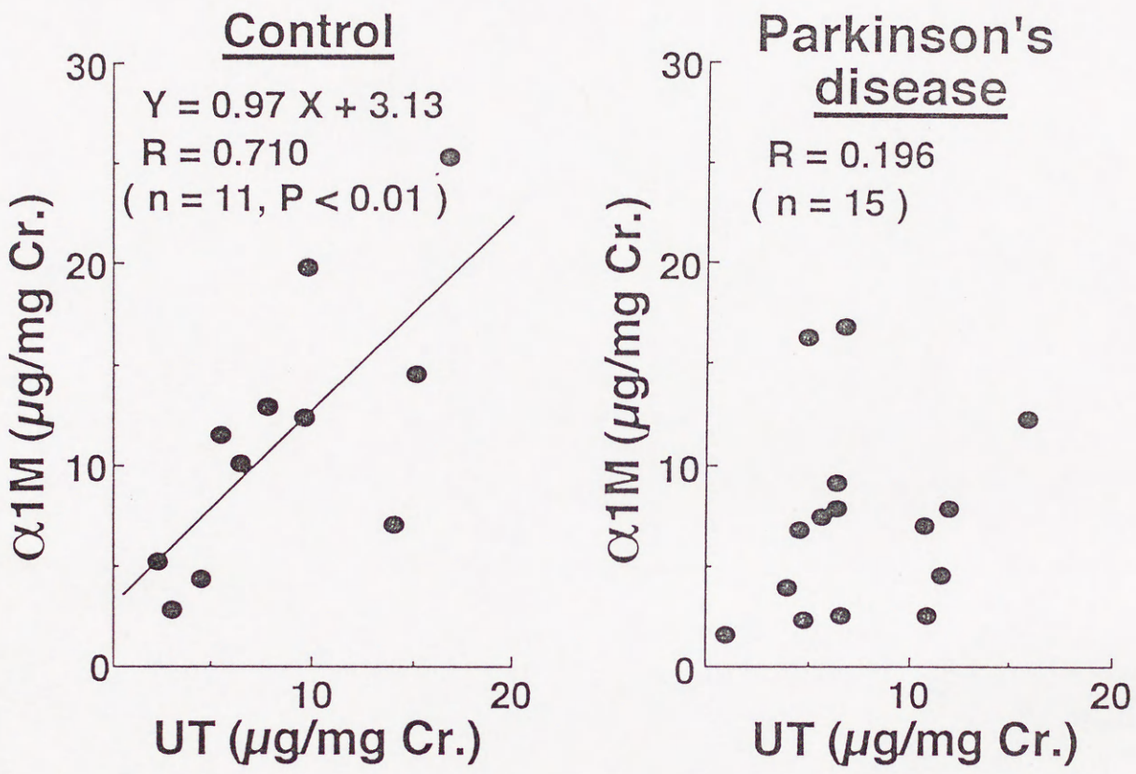
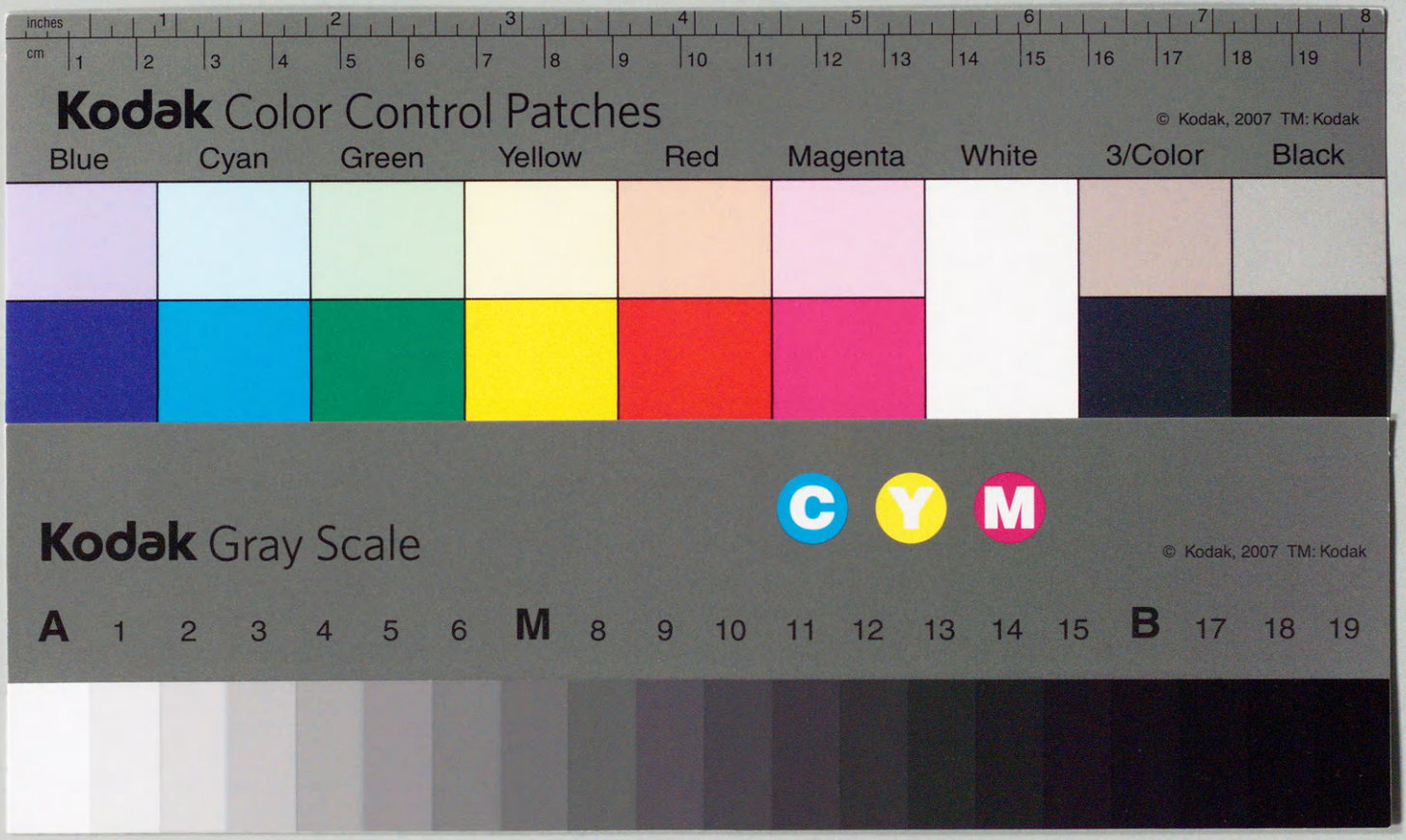


Figure 1. Correlation between urinary levels of UT and α1M in age-matched normal controls and patients with Parkinson's disease. R and n in parentheses represent the correlation coefficient and number of subjects, respectively. P value shows the significance of the regression.


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19