The validity of two novel indices of fall tendency, the 22 items fall risk index[8] and the 13 points simple screening test,[3] which were used in our previous study, have been confirmed in community-dwelling elderly, but not in geriatric outpatients. Therefore, in the present investigation, the association of these two indices with falls was also evaluated to confirm their validity in geriatric outpatients in a longitudinal study.

Methods

Patients

From 2006 to 2007, a total of 190 consecutive patients aged 65 years or older who were receiving treatment for chronic diseases, such as hypertension, dyslipidemia, diabetes and osteoporosis, who were seen every 2–4 weeks at the outpatient clinic of the Research Institute of Aging Science, Tokyo, were enrolled. All the patients were able to walk independently and their condition was stable. Patients who had acute illness or overt dementia were excluded. Anthropometric and medical information including past history of stroke, myocardial infarction, malignancy and prescribed drugs was obtained from each patient at baseline from the medical chart recorded by the physician in charge. However, 18 patients were excluded, because they were lost to follow up soon after enrolment and the medical information was not fully obtained. All prescribed drugs had not been changed in the included patients for at least 2 months before enrolment. The patients were followed up for 2 years.

Occurrence of falls

During the follow-up period, the patients and their family members responded to the annual questionnaire asking about the occurrence of falls within the past year. The questionnaire was repeated for 2 years.

Indices of fall tendency

After enrolment, the patients were examined for two indices to investigate the fall tendency. These were (i) a questionnaire of the 22 items portable fall risk index;[8] and (ii) the 13 points simple screening test to assess the fall tendency.[3]

Ethical consideration

The present study was approved by the Institutional Review Board of the Research Institute of Aging Science. We obtained written consent from all participants and/or their guardians.

Data analysis and statistical methods

Values are expressed as mean ± standard deviation. In order to analyze the relationship between falls and comorbidity or drugs, variables were compared using Student's *t*-test or χ^2-test as appropriate. Significant factors found in univariate analysis were included in multivariate logistic regression analysis to determine the association of falls with other variables. Receiver-operating curve (ROC) analysis was carried out to identify the optimal cut-off value of the number of drugs for predicting falls within 2 years. The value with the highest sum of sensitivity and specificity was used as the optimal cut-off value. Logistic regression analysis was carried out to assess the validity of the two indices of fall tendency, adjusted by age and sex. *P*-values <0.05 were considered statistically significant. Data were analyzed using JMP version 8.0.1 (SAS Institute, Cary, North Carolina, USA).

Results

Baseline medical information and two indices of fall tendency were evaluated in 172 patients (Table 1). Drugs prescribed in less than 5% of the patients are not shown. Because only patients who were in a stable condition and were able to walk independently were included, patients with Parkinson's disease, severe paresis or painful arthralgia were not included. Calcium channel blockers prescribed in the present study were all long-acting agents, and the prescribed aspirin dosage was 100 mg in all cases. Only a few patients were receiving insulin therapy, sulfonylureas, angiotensin converting enzyme inhibitors, β-blockers, α-blockers, non-steroidal anti-inflammatory drugs or anticoagulants. No patients were taking neuroleptics or antiparkinsonian drugs.

After 1 year, all patients, except for one who died of congestive heart failure, were followed up ($n = 171$, follow-up rate 99.4%). Falls occurred in 22 patients. Only a higher age was associated with falls within 1 year on univariate analysis (non-fallers: 76.4 ± 6.8 years, fallers: 81.0 ± 6.9 years, $P = 0.004$).

After another year (2 years after enrolment), one patient had died of lung cancer, and five patients were lost to follow up. A total of 165 patients were evaluated (follow-up rate 95.9%), and 10 patients had fallen during the second year; thus a total of 32 patients had fallen within 2 years. As shown in Table 2, higher age, osteoporosis, number of comorbid conditions and number of drugs were significant factors associated with falls. To determine the association of falls with these significant factors, multivariate logistic regression analysis was carried out, and as shown in Table 2, the number of drugs was the only factor that was significantly associated with falls within 2 years.

As polypharmacy was assumed to be a risk for falls within 2 years, the cut-off of the number of the drugs was analyzed. Figure 1 shows the ROC curves to define the optimal cut-off point in relation to falls within

Table 1 Characteristics and univariate analysis of association with fallers and non-fallers within 2 years and risk factors

Total		Non-fallers ($n = 133$)	Fallers ($n = 32$)	P-value (Fallers vs. Non-fallers)
Age (years)	77.0 ± 7.0	76.3 ± 6.9	80.0 ± 6.9	0.007
Body mass index (kg/cm^2)	22.7 ± 3.2	22.7 ± 3.3	22.7 ± 3.1	0.98
No. comorbid conditions	1.9 ± 1.1	1.8 ± 1.1	2.3 ± 0.9	0.009
No. drugs	3.2 ± 2.8	2.8 ± 2.7	4.9 ± 2.5	<0.0001
Female ($n = 122$)	–	72.9%	78.1%	0.66
Hypertension ($n = 106$)	–	62.4%	71.8%	0.41
Dyslipidemia ($n = 76$)	–	47.3%	40.6%	0.56
Diabetes ($n = 23$)	–	12.8%	18.8%	0.40
Osteoporosis ($n = 59$)	–	30.8%	56.3%	0.01
History of stroke ($n = 6$)	–	2.3%	9.4%	0.09
History of myocardial infarction ($n = 3$)	–	0.8%	6.3%	0.10
History of cancer ($n = 8$)	–	5.3%	3.1%	0.99
Calcium channel blocker ($n = 59$)	–	33.3%	46.9%	0.16
Angiotensin II receptor blocker ($n = 56$)	–	33.3%	37.5%	0.68
Statin ($n = 40$)	–	23.5%	28.1%	0.65
Aspirin ($n = 31$)	–	19.0%	24.1%	0.61
Bisphosphonate ($n = 9$)	–	4.6%	9.4%	0.38
H2-blocker ($n = 9$)	–	3.8%	12.1%	0.80
Proton pump inhibitor ($n = 11$)	–	5.3%	12.1%	0.23
Hypnotic ($n = 31$)	–	16.7%	28.1%	0.14

Values are expressed as mean ± SD ($n = 165$).

Table 2 Logistic regression analysis of association of falls within 2 years with age, sex, other significant factors found in univariate analysis, and polypharmacy

	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (/1 year)	1.08 (1.03–1.13)†	1.06 (0.99–1.13)	1.06 (0.99–1.13)
Sex (male = 0, female = 1)	1.39 (0.56–3.48)	0.98 (0.29–3.23)	0.75 (0.23–2.38)
Osteoporosis ($n = 0$, Y = 1)	3.12 (1.43–6.84)†	2.76 (0.92–7.38)	3.02 (0.96–6.15)
No. comorbid conditions (/disease)	1.63 (1.14–2.32)*	0.90 (0.55–1.47)	0.99 (0.62–1.56)
No. drugs (/drug)	1.29 (1.12–1.48)‡	1.30 (1.08–1.57)*	–
Five or more drugs ($n = 0$, Y = 1)	5.04 (2.25–11.3)‡	–	4.50 (1.66–12.2)†

*$P < 0.05$, †$P < 0.005$, ‡$P < 0.0005$. CI, confidence interval.

2 years: the area under the ROC was 0.731, and the optimal cut-off value of the number of drugs was five (sensitivity 0.576, specificity 0.788). Logistic regression analysis showed that taking five or more drugs was significantly associated with an increased risk of falls (odds ratio 4.5, 95% CI 1.7–12.2) after adjustment for age, sex, osteoporosis and number of comorbid conditions (Table 2).

Also, the association between falls and two indices of fall tendency was evaluated to confirm the validity of each index in geriatric outpatients. As both indices included the questionnaire asking whether patients were "taking five or more drugs," the number of drugs was excluded from this analysis because of duplication in the statistical model. As shown in Table 3, the 22 items fall risk index showed a tendency towards an association with falls within 2 years, odds ratio 1.12 (95% CI 1.00–1.26; $P = 0.05$), whereas the 13 points screening test was significantly associated with falls after adjustment for age, sex and other factors significantly associated in the univariate analysis. Therefore, these indices are considered to be good predictors of falls in geriatric outpatients, as has been shown in community-dwelling elderly subjects.

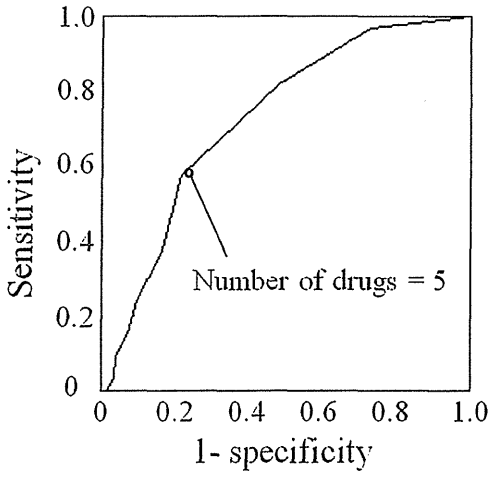

Figure 1 Receiver–operating curves to define optimal cut-off value of number of drugs at baseline in relation to falls within 2 years. Area under the curve was 0.731, optimal cut-off value of the number of drugs was five (sensitivity = 57.6%, specificity = 78.8%).

Discussion

The risk of falls has been assessed in community-dwelling elderly, and history of falls, physical ability and living environment were found to be predictors of falls. Also, in nursing home residents, cognitive function, gait disturbance and urinary incontinence are reported to be risk factors for falls,[9,10] and length of stay, disease condition, surgical procedures and some specific drugs are reported to be risk factors in hospital inpatients.[11,12]

Nevertheless, the risks in geriatric outpatients have not been sufficiently assessed, although assessment of fall risk in geriatric outpatients is important; their medical conditions or drugs might cause falls, and drugs, such as antiplatelet agents or anticoagulants, might cause critical bleeding after a fall. Also, physicians could prevent falls in their patients by giving advice during regular consultations, if risk factors are identified.

In our previous cross-sectional study assessing geriatric outpatients, polypharmacy was significantly correlated with indices of fall tendency, and the present follow-up study of geriatric outpatients showed the impact of polypharmacy on falls within 2 years. Statistical analyses showed that polypharmacy was a risk factor for falls, independent of age, sex and comorbidity.

Besides polypharmacy, several medications and comorbid conditions have been reported as risks for falls.[13–22] Among these, diabetes,[5,6] insomnia,[13] hypnotics,[13–15] antiarrhythmics[22] and antihypertensive agents[14] were not significantly associated with fall risk in the present study. Just 11 patients (45.9% of diabetic patients) were prescribed hypoglycemic agents, such as a sulfonylurea ($n = 8$) or insulin ($n = 3$), and the relatively low rate of prescription of hypoglycemic agents might have affected our result. Neither hypnotics nor antihypertensives were associated with falls. This result might be a result of the small sample size. Anti-arrhythmics were taken by just three patients (digoxin: $n = 2$, class IA anti-arrhythmic drug: $n = 1$). Other drugs, such as major tranquillizers,[14] antidepressants[17,18] and antiparkinsonian agents,[19,22] might increase fall risk; however, no patient used these drugs in the present study. In the present study, most of the patients were in a stable condition throughout the 2 years, though their drugs were changed gradually according to their medical conditions during the observation period. We only used the number of drugs at baseline for statistical analysis; however, the number of drugs increased from 3.2 ± 2.8 to 3.9 ± 3.0 during the 2 years. There were 17 patients whose number of drugs had been decreased, 70 patients not changed and 78 patients increased. The number of drugs after 2 years was also associated with falls ($P < 0.0005$). The optimal cut-off point for the number of drugs was again five (area under ROC curve 0.780, sensitivity 0.576, specificity 0.788). Furthermore, the changes in number of drugs were also associated with falls ($P < 0.05$), and the optimal cut-off point for the change in number of drugs was +1 (area under ROC curve 0.649, sensitivity 0.727, specificity 0.409).

Table 3 Logistic regression analysis of association between 2-year fall occurrences with two indices of fall tendency; 22 items fall risk index and 13 points simple screening test

	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (/year)	1.08 (1.03–1.15)**	1.06 (0.99–1.13)	1.06 (1.00–1.13)
Sex (male = 0, female = 1)	1.39 (0.56–3.48)	0.75 (0.23–2.43)	0.79 (0.24–2.56)
Osteoporosis ($n = 0$, Y = 1)	3.12 (1.43–6.84)**	2.56 (0.96–6.82)	2.61 (0.98–6.95)
No. comorbid conditions (/disease)	1.63 (1.14–2.32)*	1.24 (0.83–1.86)	1.32 (0.88–1.97)
Fall risk index (/item)	1.23 (1.11–1.37)***	1.12 (1.00–1.26)	–
Simple screening test (/point)	1.19 (1.06–1.33)**	–	1.14 (1.01–1.29)*

*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$. CI, confidence interval.

Consequently, polypharmacy, especially taking five or more drugs, should be considered a risk for falls.

There were several limitations of the present study. First, the falls were self-reported by the patients. Although all the patients had no overt dementia, they might have forgotten the incident of falling. We attempted to count the total fall occurrences in each patient; however, we could not differentiate the repeated falls in the second year from the fall occurrence in the first year. In fact, we asked 22 patients who reported falls in the first year about fall occurrence during the second year, but they did not accurately recall whether they experienced falls in the first or second year. Second, five patients were lost to follow up at 2 years for unknown reasons. The follow-up ratio was acceptable, although some of the patients might have fallen, have been no longer able to come to the clinic and moved to nursing homes. This might have slightly influenced the result. Also, the cause of falls in polypharmacy patients is not explained. Potentially inappropriate medications, which could cause adverse drug reactions, are usually seen in patients with polypharmacy, and falls might be the consequence of adverse drug reactions, such as dizziness, instability and light-headedness. Pathophysiological assessments and drug-reducing interventions are expected to elucidate the causal relationship.

Additionally, we showed that the 22-item fall risk index and its simple screening test were useful to predict falls in geriatric outpatients. Although both indices have been validated in community-dwelling elderly people, the present finding also showed their association with fall risk among geriatric outpatients. The difference of statistical significance between fall risk index and simple screening test might be a result of small sample size or the difference in the contribution of each item to total scores between the two indices. "Taking five or more drugs" accounts for only one item out of the 22-item fall risk index; in contrast, the same questionnaire accounts two points in the 13-point simple screening test. Because polypharmacy was a strong risk factor of falls in elderly outpatients in the present study, the proportion of polypharmacy in the scores might have caused the discrepancy. Taken together, it is likely that 13-point screening test was more suitable to our subjects who were taking several medicines.

In summary, the present study showed that geriatric outpatients with polypharmacy were at a high risk of falls, especially those receiving five or more drugs. Our finding might add new information for pharmacotherapy and geriatric research in elderly patients with chronic diseases. Intervention studies examining the effect of drug reduction for the prevention of falls are required in the future.

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Disclosure statement

The authors declare no conflict of interest.

References

1 Stel VS, Pluijm SM, Deeg DJ, Smit JH, Bouter LM, Lips P. A classification tree for predicting recurrent falling in community- dwelling older persons. *J Am Geriatr Soc* 2003; **51**: 1356–1364.
2 Kojima S, Furuna T, Ikeda N, Nakamura M, Sawada Y. Falls among community-dwelling elderly people of Hokkaido, Japan. *Geriatr Gerontol Int* 2008; **8**: 272–277.
3 Okochi J, Toba T, Takahashi T *et al.* Simple screening test for risk of falls in the elderly. *Geriatr Gerontol Int* 2006; **6**: 223–227.
4 Leipzig RM, Cumming RG, Tinetti ME. Drugs and falls in older people: a systematic review and meta-analysis: II. Cardiac and analgesic drugs. *J Am Geriatr Soc* 1999; **47**: 40–50.
5 Berlie HD, Garwood CL. Diabetes medications related to an increased risk of falls and fall-related morbidity in the elderly. *Ann Pharmacother* 2010; **44**: 712–717.
6 Araki A, Ito H. Diabetes mellitus and geriatric syndromes. *Geriatr Gerontol Int* 2009; **9**: 105–114.
7 Kojima T, Akishita M, Nakamura T *et al.* Association of polypharmacy with fall risk among geriatric outpatients. *Geriatr Gerontol Int* 2011; **11**: 438–444.
8 Toba K, Okochi J, Takahashi T *et al.* Development of a portable fall risk index for elderly people living in the community. *Nippon Ronen Igakkai Zasshi* 2005; **42**: 346–352. (In Japanese.)
9 Kron M, Loy S, Sturm E *et al.* Risk indicators for falls in institutionalized frail elderly. *Am J Epidemiol* 2003; **158**: 645–653.
10 van Doorn C, Gruber-Baldini AL, Zimmerman S *et al.* Dementia as a risk factor for falls and fall injuries among nursing home residents. *J Am Geriatr Soc* 2003; **51**: 1213–1218.
11 Halfon P, Eggli Y, Van Melle G, Vagnair A. Risk of falls for hospitalized patients: a predictive model based on routinely available data. *J Clin Epidemiol* 2001; **54**: 1258–1266.
12 Tanaka M, Suemaru K, Ikegawa Y *et al.* Relationship between the risk of falling and drugs in an academic hospital. *Yakugaku Zasshi* 2008; **128**: 1355–1361.
13 Ensrud KE, Blackwell TL, Redline S *et al.* Sleep disturbances and frailty status in older community-dwelling men. *J Am Geriatr Soc* 2009; **57**: 2085–2093.
14 Leipzig RM, Cumming RG, Tinetti ME. Drugs and falls in older people: a systematic review and meta-analysis: I. Psychotropic drugs. *J Am Geriatr Soc* 1999; **47**: 30–39.
15 Woolcott JC, Richardson KJ, Wiens MO *et al.* Meta-analysis of the impact of 9 medication classes on falls in elderly persons. *Arch Intern Med* 2009; **169**: 1952–1960.

16 Tinetti ME, Speechley M, Ginter SF. Risk factors for falls among elderly persons living in the community. *N Engl J Med* 1988; **319**: 1701–1707.
17 Kelly KD, Pickett W, Yiannakoulias N *et al.* Medication use and falls in community-dwelling older persons. *Age Ageing* 2003; **32**: 503–509.
18 Thapa PB, Gideon P, Cost TW, Milam AB, Ray WA. Antidepressants and the risk of falls among nursing home residents. *N Engl J Med* 1998; **339**: 875–882.
19 Bloem BR, Steijns JA, Smits-Engelsman BC. An update on falls. *Curr Opin Neurol* 2003; **16**: 15–26.
20 Arai H, Akishita M, Teramoto S *et al.* Incidence of adverse drug reactions in geriatric units of university hospitals. *Geriatr Gerontol Int* 2005; **5**: 293–297.
21 Iwata M, Kuzuya M, Kitagawa Y, Suzuki Y, Iguchi A. Underappreciated predictors for postdischarge mortality in acute hospitalized oldest-old patients. *Gerontology* 2006; **52**: 92–98.
22 Baranzini F, Diurni M, Ceccon F *et al.* Fall-related injuries in a nursing home setting: is polypharmacy a risk factor? *BMC Health Serv Res* 2009; **9**: 228.

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REFERENCES

1. Sikkes SAM, Visser PJ, Knol DL et al. Do instrumental activities of daily living predict dementia at 1- and 2-year follow-up? Findings from the Development of Screening guidelines and diagnostic Criteria for Predementia Alzheimer's disease study J Am Geriatr Soc 2011;59:2273–2278.
2. Folstein MF, Folstein SE, McHugh PE. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–189.
3. Nasreddine ZS, Phillips NA, Bédirian V et al. The Montreal Cognitive Assessment (MoCA): A brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695–699.
4. Nouri FM, Lincoln NB. An extended activities of daily living scale for stroke patients. Clin Rehabil 1987;1:301–305.
5. Regal P. Antithyroid antibodies, cognition and IADL in the elderly. Int J Geriatr Psychiatry 2012;27, in press.

FACTORS ASSOCIATED WITH PROLONGED HOSPITAL STAY IN A GERIATRIC WARD OF A UNIVERSITY HOSPITAL IN JAPAN

To the Editor: We read with interest the article by Lakhan and colleagues,[1] which showed the high prevalence and worsening of geriatric syndrome during acute care hospitalization. Because falls, incontinence, impairment in activities of daily living (ADLs), and other geriatric syndrome components increase the care burden and limit discharge planning in acute care hospitals, geriatric syndrome might cause prolonged hospital stays. A prolonged hospital stay is one of the major determinants of medical cost and is thus a serious problem in geriatric medicine. Previous studies have shown that clinical events during hospitalization,[2,3] basic ADLs,[4] and nonmedical factors such as delayed transfer to a nursing facility or disagreement on the discharge plan among family members[5] are risk factors for prolonged hospital stay. Furthermore, because older adults have multiple comorbid conditions and are susceptible to adverse drug reactions (ADRs), these factors might be related to length of hospital stay. To test this hypothesis, the association between geriatric conditions such as geriatric syndrome, ADLs, and ADRs and prolonged hospital stay were comprehensively investigated using the database of the geriatric ward of the University of Tokyo Hospital from 1995 to 2010. The ethics committee of the Graduate School of Medicine, University of Tokyo approved this study.

All records of patients aged 65 and older from 1995 to 2010 were reviewed. Data on length of stay, acute hospitalization, ADRs, body mass index (BMI), number of diseases and drugs, geriatric syndrome, and Barthel Index were collected. Twenty-three components of geriatric syndrome such as falls, cognitive impairment, urinary incontinence, constipation, and insomnia were included in the analysis. Records lacking information on any of the variables were excluded. Cases of scheduled short-term hospitalization were excluded. Finally, the records of 1,616

Table 1. Characteristics of Study Patients and Analyses for Length of Hospital Stay (N = 1,616)

Characteristic	Value	Univariate Analysis (R or Hospital Stay, Days, Mean ± SD)	Standardized Regression Coefficient
Age, mean ± SD	78.3 ± 7.0	0.001	−0.099[d]
Sex, *n* (%)			
Female	778 (48.1)	26.8 ± 20.2	
Male	838 (51.9)	27.6 ± 24.6[a]	
Acute hospitalization, *n* (%)			
Yes	300 (18.5)	26.2 ± 21.0	
No	1,316 (81.5)	31.8 ± 28.2[a,d]	
Adverse drug reaction, *n* (%)			
Yes	190 (11.8)	26.4 ± 19.5	0.078[c]
No	1,426 (88.2)	33.3 ± 38.1[a,d]	
Body mass index, kg/m^2, mean ± SD	22.0 ± 4.1	−0.59[d]	−0.062[b]
Barthel Index (points out of 100), mean ± SD	83.1 ± 26.1	−0.178[d]	−0.13[d]
Number of diseases, mean ± SD	5.3 ± 2.3	1.43[c]	0.082[c]
Number of drugs, mean ± SD	6.8 ± 3.6	0.411[b]	–
Number of geriatric syndrome components, mean ± SD	4.6 ± 3.6	1.66[d]	0.19[d]

All data were collected soon after admission. For sex, acute hospitalization, and adverse drug reactions, a simple *t*-test was performed for univariate analysis, and values are expressed as mean ± standard deviation (SD).

[a]*P*-values are for comparison to female or no. Pearson correlation coefficients (R) are shown for the remaining factors in univariate analysis. All variables shown were included in stepwise regression analysis, and factors significantly associated were analyzed in multiple regression analysis (coefficient of determination = 0.32).

[b]$P < .05$.

[c]$P < .005$.

[d]$P < .001$.

patients were analyzed (mean age 78.3 ± 7.0, 52% male). All data were obtained soon after admission. Values are expressed as means ± standard deviations and were analyzed using JMP version 9.0.2 (SAS Institute, Inc., Cary, NC). $P < .05$ was considered statistically significant.

Mean length of stay was 27.3 ± 22.6 days (range 1–322 days). The results of univariate and multivariate analyses for length of stay are shown in Table 1. Multiple stepwise regression analysis showed that ADRs, number of diseases, and number of geriatric syndrome components were positively associated with longer hospital stay, whereas age, BMI, and Barthel Index were negatively associated. The number of geriatric syndrome components was significantly associated with hospital stay independent of number of diseases.

The present analysis demonstrated that geriatric factors such as ADRs, multiple diseases, low BMI, ADL dependence, and number of geriatric syndrome components were associated with longer hospital stay in a large group. The finding that ADRs are a risk for prolonged hospital stay is consistent with a previous report,[6] and ADL dependence has been reported as a risk in a smaller group.[4] Furthermore, the number of geriatric syndrome components and undernutrition were risk factors for prolonged hospital stay in a large-scale study. Frailty, which is also known to be a risk factor,[7] was not examined independently in the present study, but ADL dependence and undernutrition, both of which are major components of frailty, were found to be risk factors, so it is reasonable to assume that frailty was associated with length of hospital stay in the current cohort as well. The present study revealed that the accumulation of geriatric syndrome components was a risk factor for prolonged hospital stay independent of multiple diseases and, presumably, frailty. Thus, geriatric syndrome should be comprehensively managed during hospitalization. The reason for the negative association between age and length of stay is unclear, but the presence of young-old patients with disability or complicated conditions on the geriatric ward might have influenced the results.

In summary, the present study provides new insight into the significance of geriatric conditions in relation to prolonged hospital stay in older adults. ADL dependence, undernutrition, ADRs, and geriatric syndrome should be carefully assessed and interventions provided when caring for older inpatients.

Taro Kojima, MD
Masahiro Akishita, MD, PhD
Yumi Kameyama, MD, PhD
Kiyoshi Yamaguchi, MD, PhD
Hiroshi Yamamoto, MD, PhD
Masato Eto, MD, PhD
Yasuyoshi Ouchi, MD, PhD
Department of Geriatric Medicine
Graduate School of Medicine
University of Tokyo, Tokyo, Japan

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REFERENCES

1. Lakhan P, Jones M, Wilson A et al. A prospective cohort study of geriatric syndromes among older medical patients admitted to acute care hospitals. J Am Geriatr Soc 2011;59:2001–2008.
2. Nobili A, Licata G, Salerno F et al. Polypharmacy, length of hospital stay, and in-hospital mortality among elderly patients in internal medicine wards. The REPOSI Study. Eur J Clin Pharmacol 2011;67:507–519.
3. Hauck K, Zhao X. How dangerous is a day in hospital? A model of adverse events and length of stay for medical inpatients. Med Care 2011;49:1068–1075.
4. Umegaki H, Ando F, Shimokata H et al. Factors associated with long hospital stay in geriatric wards in Japan. Geriatr Gerontol Int 2003;3:120–127.
5. Foer D, Ornstein K, Soriano TA et al. Nonmedical factors associated with prolonged hospital length of stay in an urban homebound population. J Hosp Med 2012;7:73–78.
6. Hoonhout LH, de Bruijne MC, Wagner C et al. Nature, occurrence and consequences of medication-related adverse events during hospitalization: A retrospective chart review in the Netherlands. Drug Saf 2010;33:853–864.
7. Satish S, Winograd CH, Chavez C et al. Geriatric targeting criteria as predictors of survival and health care utilization. J Am Geriatr Soc 1996; 44:914–921.

ACTIVITIES OF DAILY LIVING RATHER THAN DEPRESSIVE SYMPTOMS INCREASE THE RISK OF MORTALITY IN JAPANESE COMMUNITY-DWELLING ELDERLY PEOPLE: A 4-YEAR LONGITUDINAL SURVEY

To the Editor: The article entitled "Depressive Symptoms Increase the Risk of Mortality in Older Mexican Community-Dwelling Adults" by Piña-Escudero et al.[1] deeply impressed us. Although it has been shown that older adults with depressive symptoms (DSs) have fewer quality-adjusted life years than those with chronic medical conditions,[2] Piña-Escudero et al. in their 2-year longitudinal study, showed that DSs increase mortality risk regardless of multiple covariates such as medical conditions and disability in activities of daily living (ADL). Similarly, results of a meta-analysis of 25 studies suggest that depression increases the risk of mortality,[3] although those studies did not assess ADL in detail. The risk of mortality in Japanese community-dwelling elderly people is reported herein, focusing on DSs and ADLs in a 4-year longitudinal survey.

The study population included 1,818 community-dwelling individuals aged 65 and older in Tosa Town, Japan; 1,600 (88.0%) participants who completed self-reported geriatric questionnaires in 2006 were included in the study. The questionnaires consisted of questions on ADLs and the 15-item Geriatric Depression Scale (GDS-15).[4] For ADL assessment, participants rated their

RELATIONSHIP BETWEEN TESTOSTERONE AND COGNITIVE FUNCTION IN ELDERLY MEN WITH DEMENTIA

To the Editor: A decrease in sex hormones with aging has been reported to be related to psychosomatic disorders such as late-onset hypogonadism syndrome, frailty, and cognitive impairment in adult men.[1] For example, a community-based cross-sectional study has shown that elderly men with a lower blood concentration of bioavailable testosterone have more-severe impairment of cognitive function.[2] Moreover, a longitudinal study indicated that serum free testosterone (FT) concentration could predict memory performance and cognitive status in elderly men,[3] but it is unknown whether lower testosterone concentration is related to cognitive impairment in individuals with dementia, because the previous studies primarily focused on a healthy community-based population. Also, few studies have addressed the relationship between testosterone and cognitive function in elderly Japanese men.

One recent cross-sectional study showed that total testosterone and FT concentration were associated with activities of daily living (ADLs) in institutionalized elderly men.[4] This study also revealed that a relationship between testosterone and cognitive function could be found even in institutionalized elderly men with physical or neuropsychiatric dysfunction. Thus, whether lower testosterone concentration is related to deterioration of ADL in elderly men with cognitive impairment was longitudinally investigated.

Fifty-two male outpatients attending the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were recruited (mean age 77.0 ± 5.5, range 65–87). Participants' clinical backgrounds were hypertension, 48.9%; diabetes mellitus, 12.2%; and dyslipidemia, 38.1%. None had a history of stroke. Comprehensive geriatric assessment was performed based on basic ADLs (Barthel Index),[5] instrumental ADLs (Lawton and Brody IADLs, 0–5 points in men),[6] cognitive function (Mini-Mental State Examination (MMSE)),[7] mood (Geriatric Depression Scale (GDS), 15 items),[8] and vitality (Vitality Index, 10-point scale).[9] This assessment was repeated 1, 2, and 3 years after baseline assessment at the first visit to the clinic. At the first visit, blood was drawn after an overnight fast and FT concentration was measured using radioimmunoassay. FT values ranged from 1.0 to 53.0 pmol/L (mean ± SD 30.4 ± 11.0 pmol/L). Participants were classified into three groups according to tertile according to the baseline FT value (Figure 1), and the parameters from the comprehensive geriatric assessment were compared between groups and visits. Statistical data were analyzed using SPSS version 17.0 (SPSS, Inc., Chicago, IL). One-way analysis of variance (ANOVA) was applied for comparisons between groups, and the Fisher post hoc test was applied when significant ($P < .05$). One-way repeated ANOVA was used for comparisons between baseline and the 1-, 2-, and 3-year visits, and the Fisher post hoc test was applied when significant ($P < .05$).

There were no significant differences between groups in age (high, 75.3; middle, 76.6; low, 79.0), basic ADLs (high, 96.9; middle, 99.1; low, 95.3 points), MMSE (high, 23.2; middle, 25.1; low, 23.1 points), GDS-15 (high, 5.1; middle, 4.1; low, 4.6 points), and Vitality Index (high, 9.1; middle, 9.1; low, 8.8 points) at baseline, whereas IADLs tended to be lower (high, 4.1; middle, 4.1; low, 3.4 points, $P = .06$) in the low FT tertile group than in the other groups.

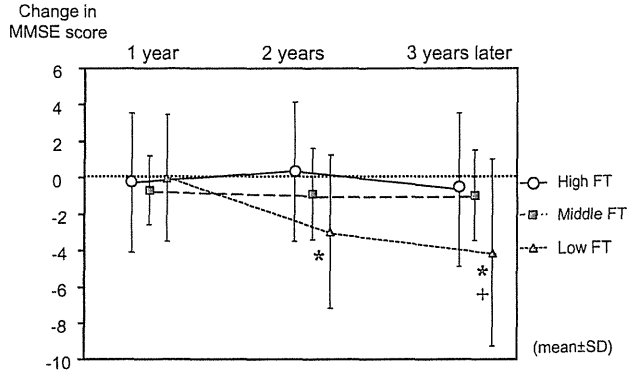

Figure 1. Change in Mini-Mental State Examination (MMSE) score according to tertile of serum free testosterone (FT) level in men. FT tertile: high, >36.1 pmol/L, n = 17; middle, 29.1–35.4 pmol/L, n = 17; low, <28.8 pmol/L, n = 18. *$P < 0.05$ vs highest FT group, +$P < 0.05$ vs middle FT group.

At the 1-year visit, there was no difference in change in MMSE score from baseline between the groups, although the decrease in MMSE score was larger in the low FT tertile group than in the middle and high tertile groups at the 2- and 3-year visits (Figure 1). Also, MMSE scores were lower in the low FT tertile group at the 2- ($P = .009$) and 3-year ($P < 0.001$) visits than at baseline, whereas they were not lower in the middle and high tertile groups. In contrast, there was no such trend in basic ADLs, IADLs, GDS scores, and Vitality Index.

Multiple regression analysis was performed with a decrease in MMSE score as a dependent variable and age; ADLs; body mass index; presence of hypertension, diabetes mellitus, or hyperlipidemia; and FT concentration as independent variables to consider factors affecting cognitive impairment, according to a previous report.[4] Blood FT concentration was found to be an independent predictor of decrease in MMSE score at the 3-year visit ($\beta = 0.492$, $P = .02$).

A number of investigations support the biological plausibility of a protective effect of testosterone against cognitive dysfunction. The present findings from memory clinic outpatients are consistent with previous findings observed in elderly community-based men, showing a relationship between FT concentration and cognitive performance.[3] Furthermore, the present findings indicate that a lower FT concentration could lead to a faster decline in cognitive function in elderly Japanese men who already show cognitive impairment. This study provides fundamental data for the future study of hormone replacement therapy for cognitive decline in elderly adults with low FT.

Kumiko Nagai, PhD
Shigeki Shibata, MD, PhD
Yoshio Kobayashi, MD
Yukiko Yamada, MA
Sayaka Kimura, MA

Ayako Machida, ST
Koichi Kozaki, MD, PhD
Department of Geriatric Medicine, School of Medicine
Kyorin University, Tokyo, Japan

Masahiro Akishita, MD, PhD
Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Kenji Toba, MD, PhD
National Center for Geriatrics and Gerontology
Aichi, Japan

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REFERENCES

1. Ulubaev A, Lee DM, Purandare N et al. Activation effects of sex hormones on cognition in men. Clin Endocrinol 2009;71:607–623.
2. Yaffe K, Lui LY, Zmuda J et al. Sex hormones and cognitive function in older men. J Am Geriatr Soc 2002;50:707–712.
3. Moffat SD, Zonderman AB, Metter EJ et al. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab 2002;87:5001–5007.
4. Fukai S, Akishita M, Yamada S et al. Association of plasma sex hormone levels with functional decline in elderly men and women. Geriatr Gerontol Int 2009;9:282–289.
5. Mahoney FI, Barthel DW. Functional evaluation: Barthel Index. Md State Med J 1965;14:61–65.
6. Lawton MP, Brody EM. Assessment of older people, self-maintaining and instrumental activities of daily living. Gerontologist 1969;9:179–186.
7. Folstein MF, Folstein SE, McHugh PR. 'Mini-Mental State'. A practical method for grading the cognitive state of patients for clinician. J Psychiatr Res 1975;12:189–198.
8. Yesavage JA. Geriatric Depression Scale. Psychopharmacol Bull 1988;24: 709–711.
9. Toba K, Nakai R, Akishita M et al. Vitality Index as a useful tool to assess elderly with dementia. Geriatr Gerontol Int 2002;2:23–29.

BASELINE INSTRUMENTAL ACTIVITIES OF DAILY LIVING AND INCIDENT DEMENTIA

To the Editor: Sikkes et al.[1] have written an important paper showing that individuals without dementia with impairment in at least one of nine instrumental activities of daily living (IADLs) at baseline had a significantly higher incidence of dementia at 12 months (24.4%) than individuals without IADL impairment at baseline (16.7%) (P = .04). Their 531 participants who were followed for 12 months were relatively young (mean age 69.6), so it was decided to duplicate their study from prospective data from the Wyong Hospital Memory Clinic, 100 km north of Sydney. From 415 individuals attending a memory clinic, community-dwelling individuals aged 60 and older who were free of dementia at baseline and had a Mini-Mental State Examination score (MMSE[2]) of 25 to 30 and a follow-up MMSE and Montreal Cognitive Assessment (MoCA), range 0 (worst) to 30 (best)[3] at 12 months were selected in a consensus conference of a geriatrician (PJ) and a clinical nurse consultant (EH). Each individual's family rated IADLs on the Nottingham scale,[4] which ranged from 0 (worst) to 22 (best). Twenty-two of 82 (27%) converted to dementia at 12 months, compared with Sikkes conversion rate of 20.8% at 24 months—the most likely reason for this difference was that mean age (79.1) was 9.5 years older than theirs (69.6). Stats Direct Version 2.7.8b (StatsDirect Ltd, Altrincham, UK) from November 2011 was used to compare converters and nonconverters. Mean age of the 22 converters at baseline was significantly higher that that of the 60 nonconverters (82.0 ± 5.8 vs 78.0 ± 6.8, P < .01), mean IADL score at baseline was significantly lower (13.1 ± 5.3 vs 16.1 ± 4.0, P = .0236), MMSE score at baseline was by definition lower (25.6 ± 0.73 vs 27.5 ± 1.50, P < .001), and MoCA score at baseline was lower (19.2 ± 3.5 vs 22.8 ± 3.9, P < .001). At 12 months, IADL (11.4 ± 5.6 vs 15.4 ± 4.5, P = .004), MMSE score (21.6 ± 4.5 vs 27.4 ± 1.6, P < .001), MoCA (16.8 ± 3.6 vs 22.8 ± 4.2, P < .001) remained significantly lower in converters.

The Nottingham IADL covers seven of the nine IADL items that Sikkes used, excluding medications and finances. Women are more likely than men to perform five of the Nottingham IADL items unless the men live alone with no home care services: cleaning the kitchen, making a hot snack, washing small items of clothing, doing a full clothes wash, and doing housework.

Although the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, criteria for dementia include a decline in social and occupational function, there is a surprising lack of research into IADLs as a predictor of incident dementia. This is an important topic for future research and ongoing studies are being conducted in three cohorts: Wyong Memory Clinic; general medical inpatients with delirium or subsyndromal delirium—a prospective randomized controlled trial, Central Coast Australia Delirium Intervention Study; and PhD study, PR DEFEAT DELIRIUM, in outpatients at high risk for incident delirium. One study[5] with 255 community-dwelling individuals attending a memory clinic who were followed an average of 13 months has been published. The 11.4% of participants with antithyroid antibodies had similar outcomes at 12 months with respect to IADLs, decline in IADLs, MMSE and MoCA scores, and transfer to residential care.

Paul Regal, MD
Department of Geriatric Medicine

Eileen Heatherington, RN
Dementia Advisory Service, Wyong Hospital, Kanwal
New South Wales, Australia

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Akira Kanno, Yutaka Suzuki, Masayuki Minami, Katsuhiko Ogawa, Minoru Oishi and Satoshi Kamei
Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan

References

1 Luft BJ, Chua A. Central nervous system toxoplasmosis in HIV pathogenesis, diagnosis, and therapy. *Curr Infect Dis Rep* 2000; **2**: 358–362.
2 Luft BJ, Hafner R, Korzun AH *et al.* Toxoplasmic encephalitis in patients with the acquired immunodeficiency syndrome. Members of the ACTG 077p/ANRS 009 Study Team. *N Engl J Med* 1993; **329**: 995–1000.
3 Meada T, Saito T, Takeuchi T, Asai T. Evaluation of a nested-pCR to detect 18S rDNA for the diagnosis of toxoplasmic meningoencephalitis. *Kansenshogaku Zasshi* 2005; **79**: 543–548.
4 Ciricillo SF, Rosenblum ML. Use of CT and MR imaging to distinguish intracranial lesions and to define the need for biopsy in AIDS patients. *J Neurosurg* 1990; **73**: 720–724.
5 Miguel J, Champalimaud JL, Borges A *et al.* Cerebral toxoplasmosis in AIDS patients, CT and MRI images and differential diagnostic problems. *Acta Med Port* 1996; **9**: 29–36.
6 Masamed R, Meleis A, Lee EW, Hathout GM. Cerebral toxoplasmosis: case review and description of a new imaging sign. *Clin Radiol* 2009; **64**: 560–563.
7 Pawelec G, Effros RB, Caruso C, Remarque E, Barnett Y, Solana R. T cells and aging (update February 1999). *Front Biosci* 1999; **4**: D216–D269.

High risk of adverse drug reactions in elderly patients taking six or more drugs: Analysis of inpatient database

Dear Editor,

Polypharmacy is frequently seen in elderly patients, largely because of the existence of multiple comorbid conditions. All medications have the potential for harm as well as benefit, and thus, physicians must make difficult trade-offs between both sides of guideline-directed care.[1,2] Some drugs are reported to increase adverse drug reactions (ADR), and have been listed as potentially inappropriate medications (PIM), which should not be used generally in elderly patients.[3–5] However, it is still complicated for general practitioners to check PIM for each patient. As polypharmacy is a well-known risk for ADR,[6,7] and the frequency of PIM use rises sharply according to the number of drugs,[7] the optimal number of drugs defining polypharmacy might be of substantial help for physicians. Therefore, we aimed to determine the cut-off number of drugs in relation to ADR using the inpatient database of our geriatric department.

All records of patients aged 65 years or older who were admitted to the Department of Geriatric Medicine, The University of Tokyo Hospital, Tokyo, Japan, from 1995 to 2010 were reviewed. Retrospective use of the patient database was approved by the ethics committee of The University of Tokyo. Records lacking information on ADR or the number of drugs and patients taking no drugs were excluded. Finally, we analyzed the records of 2412 patients (mean ± SD age = 78.7 ± 7.3 years, male 51.3%). ADR was defined as unintended or undesired harmful effects presumably caused by drugs. The occurrence of ADR was assessed before discharge by the physician in charge, and other data were obtained soon after admission. Odds ratios with 95% confidence intervals for ADR were obtained by logistic regression analysis. The receiver operating characteristic (ROC) curve was assessed to define the optimal number of drugs in relation to ADR. Data were analyzed using JMP version 9.0.2 (SAS Institute, Cary, NC, USA).

The number of prescribed drugs per patient was 6.6 ± 3.6 (mean ± SD; range = 1–30), and ADR were observed in 252 patients (10.5%). Patients with ADR were taking more drugs than those without ADR (7.6 ± 3.8 *vs* 6.4 ± 3.5 drugs, $P < 0.0001$ by unpaired *t*-test). ADR was significantly associated with the number of drugs in unadjusted and age- and sex-adjusted logistic regression analysis (data not shown). When ADR were analyzed according to the number of drugs by quintile, the odds ratio of ADR was significantly higher in the groups taking six or more drugs (Fig. 1). Furthermore, ROC analysis showed that the optimal cut-off number of drugs was six, although the sensitivity of 0.560 and specificity of 0.710 were not high, with a small area under the curve of 0.591.

Previously, elderly outpatients taking five to eight drugs were reported to be at greater risk of ADR-related hospitalization than those taking zero to four drugs.[6] Also, we have reported that taking five or more drugs is a risk factor for falls in outpatients.[8] Taking these findings together, it might be reasonable to consider six or more drugs as the cut-off of polypharmacy in terms of ADR in elderly patients. The present study had some limitations; the results were obtained from inpatients managed by geriatricians, and thus might not extend to general outpatients. Next, this database did not have information for types of ADR; so they could not be clarified in detail in the present. According to our previous study, hematological, neurological and

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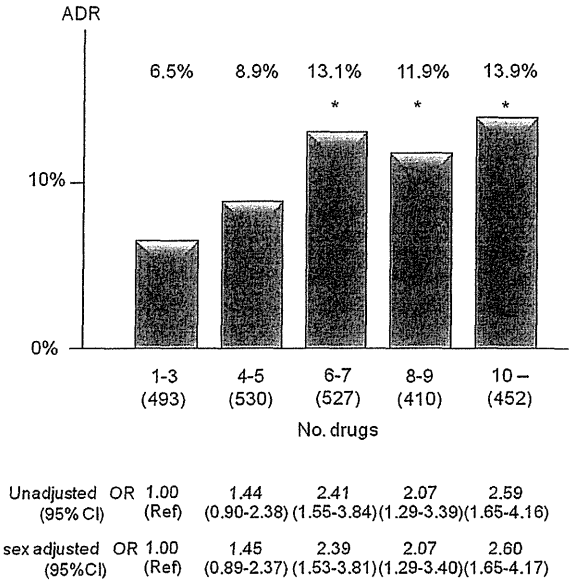

Figure 1 Frequency of adverse drug reactions according to quintile of number of prescribed drugs. Unadjusted and age–sex adjusted odds ratios (95% confidence interval) of adverse drug reactions are shown. $^*P < 0.05$ versus one to three drugs. OR, odds ratio.

cardiovascular events were reported to be more frequent than ADR in elderly inpatients,[9] and so, these are possibly the major types in the present study. Also, ROC analysis did not fit well for the present cohort.

In summary, the present study provided the cut-off number of drugs for screening of elderly patients at high risk of ADR. Prospective studies and intervention studies examining the effect of drug reduction on ADR and comorbid conditions are required to confirm this finding.

Taro Kojima, Masahiro Akishita, Yumi Kameyama, Kiyoshi Yamaguchi, Hiroshi Yamamoto, Masato Eto and Yasuyoshi Ouchi
Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

References

1 Fried TR, Tinetti ME, Iannone L. Primary care clinicians' experiences with treatment decision making for older persons with multiple conditions. *Arch Intern Med* 2011; **171**: 75–80.
2 Steinman MA, Handler SM, Gurwitz JH *et al.* Beyond the prescription: medication monitoring and adverse drug events in older adults. *J Am Geriatr Soc* 2011; **59**: 1513–1520.
3 Fick DM, Cooper JW, Wade WE *et al.* Updating the beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. *Arch Intern Med* 2003; **163**: 2716–2724.
4 Gallagher P, Ryan C, Byrne S *et al.* STOPP (Screening Tool of Older Person's Prescriptions) and START (Screening Tool to Alert doctors to Right Treatment). Consensus validation. *Int J Clin Pharmacol Ther* 2008; **46**: 72–83.
5 Akishita M, Arai H, Arai H *et al.* Survey on geriatricians' experiences of adverse drug reactions caused by potentially inappropriate medications: commission report of the Japan Geriatrics Society. *Geriatr Gerontol Int* 2011; **11**: 3–7.
6 Marcum ZA, Amuan ME, Hanlon JT *et al.* Prevalence of unplanned hospitalizations caused by adverse drug reactions in older veterans. *J Am Geriatr Soc* 2012; **60**: 34–41.
7 Steinman MA, Landefeld CS, Rosenthal GE *et al.* Polypharmacy and prescribing quality in older people. *J Am Geriatr Soc* 2006; **54**: 1516–1523.
8 Kojima T, Akishita M, Nakamura T *et al.* Association of polypharmacy with fall risk among geriatric outpatients. *Geriatr Gerontol Int* 2011; **11**: 438–444.
9 Toba K, Akishita M, Mizuno Y *et al.* Adverse drug reaction in the elderly. *Nihon Ronen Igakkai Zasshi* 1999; **36**: 181–185.

C-kit-positive acute myelogenous leukemia effectively treated with imatinib: A case report and review of the literature

It is highly advisable to choose a strategy to improve the quality of life (QOL), rather than a curative strategy, such as conventional chemotherapy, for very elderly patients with acute myelogenous leukemia (AML). Molecular targeted therapy might also be considered as an important strategy to take into account.[1]

An 88-year-old man was referred to Juntendo University Urayasu Hospital in Chiba, Japan, because of fever and headache in April 2004. The spleen was enlarged to 5 cm below the left costal margin. White blood cell (WBC) count was $61.1 \times 10^4/\mu L$, with 29% blasts and 6.5% basophils. Other data were hemoglobin (Hb) 10.6 g/dL, platelet (plt) $41.0 \times 10^4/\mu L$, lactate dehydrogenase (LDH) 685 IU/L, uric acid (UA) 10.0 mg/dL and C-reactive protein (CRP) 14.6 mg/dL. Bone marrow was myeloid hyperplasia with 27% blasts. Flow cytometer showed that the leukemic cells were positive for myeloperoxidase, CD7, CD13, CD15, CD33, CD34 and c-kit (CD117). Because the leukocytosis with blasts, mild basophilia and splenomegaly resembled blast crisis of chronic myeloid leukemia, and furthermore the patient was very old, imatinib 600 mg daily was tried. Fortunately, imatinib was effective before chromosome analysis later showed trisomy 8. Although the rate of blasts in the peripheral WBC was almost constant, the number of WBC decreased and red blood cells transfusion (RBCT) was not required soon. The patient could leave hospital on day 28 and he had a good QOL. On day 90, the WBC count was 5000/μL with 28% blasts, and Hb and plt were stable; furthermore, the spleen was not palpable. Although generalized edema and pleural effusion occurred as side-effects of imatinib on day 110, they improved with furosemide. However, on day 130, the number of WBC gradually increased,

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Gastrointestinal hemorrhage and antithrombotic drug use in geriatric patients

Dear Editor,

Recent guidelines recommend the aggressive use of antithrombotic medications in patients at high risk of thrombotic events. Although the risk of thrombosis increases with age, critical bleeding related to antithrombotic drug use is frequently seen in older patients.[1] Thus, guideline-directed use of antithrombotic medications might cause more harm than benefits among older patients with multiple comorbid conditions.[2,3] To increase the benefit-to-harm ratio, geriatricians might take care to stratify the risks and totally manage the patients. We hypothesized that such geriatricians' approaches lead to harmless use of antithrombotic medications. For this purpose, we carried out a case–control study to investigate the association between gastrointestinal hemorrhage and antithrombotic drug use.

We analyzed the inpatient registry of the Department of Geriatric Medicine, University of Tokyo Hospital between 1996 and 2007 (2249 patients) to identify patients ≥60 years-of-age who were admitted to the department as a result of gastrointestinal hemorrhage. The database was searched using the keywords of gastrointestinal hemorrhage, melena, hematemesis and anemia. Then, medical records of the extracted patients were reviewed. Finally, a total of 47 patients were defined to fulfil the criteria. Next, using risk-set sampling, we selected four controls per case matched for age, sex and the timing of hospitalization from the same inpatient registry. The data were obtained on prescriptions of antithrombotic drugs (aspirin, warfarin, cilostazol and ticlopidine) and anti-ulcer drugs (proton pump inhibitors and H2 blockers), and comorbid conditions.

Among the cases, causes of gastrointestinal hemorrhage were ulcer (48.9%), cancer (8.5%), ischemic colitis (6.3%), colon diverticulum (4.2%), Mallory–Weiss syndrome (4.2%) and hemorrhoid (2.1%), and 21.2% remained uncertain. As shown in Table 1, 17 cases and 71 controls were taking antithrombotic drugs. Of them, aspirin was most frequently prescribed both in case and control groups. There was no significant difference between case and control groups in the prescription rate of antithrombotic drugs ($\chi^2 = 0.20$, $P = 0.65$) and that of aspirin ($\chi^2 = 0.43$, $P = 0.51$). Furthermore, unadjusted logistic regression analyses showed that antithrombotic drug use and antiulcer drug use was not associated with gastrointestinal hemorrhage. The odds ratio of antithrombotic drug use for gastrointestinal hemorrhage was 0.91 (95% CI 0.46–1.81) after adjustment by age, sex and anti-ulcer drug use. Exclusion of the patients with cancer-related hemorrhage did not fundamentally influence the analytical results (data not shown).

This small case–control study showed no association of admission as a result of gastrointestinal hemorrhage with the use of antithrombotic drugs or aspirin among older patients. As most of the patients were managed by geriatricians in our department, the finding might be limited to the particular facility or cohort, but might not be extended to the general population. It is suggested, however, that geriatricians can make an appropriate decision on the indication and management of antithrombotic drugs for older patients. Although no studies have shown comparable findings in terms of gastrointestinal bleeding, geriatric evaluation and management has been reported to be effective to reduce serious adverse drug events.[4] A recent review on the management of antiplatelet agents[5] also recommended comprehensive strategies to reduce the risk of hemorrhagic complications. Prospective studies with a large sample size are required to confirm this issue. Nevertheless, it is certain that the use of antithrombotic

Table 1 Age, sex and medication use in case and control subjects, and unadjusted odds ratios for gastrointestinal hemorrhage

	Cases ($n = 47$)	Controls ($n = 189$)	Odds ratio (95% CI)
Age (years)	78 ± 10	77 ± 9	1.02 (0.98–1.06)
Men (women = 0, men = 1)	29 (61.7%)	120 (63.5%)	0.93 (0.48–1.79)
Antithrombotic drugs (no = 0, yes = 1)	16 (34.0)	71 (37.5)	0.86 (0.44–1.68)
Aspirin (no = 0, yes = 1)	10 (21.3)	49 (25.9)	0.77 (0.36–1.67)
Anti-ulcer drugs (no = 0, yes = 1)	18 (38.2)	45 (23.8)	0.67 (0.35–1.29)

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medi cations should be carefully determined by considering the risk/benefit balance of each patient.

Yoko Yamada, Masato Eto, Hiroshi Yamamoto, Masahiro Akishita and Yasuyoshi Ouchi
Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

References

1 Garcia Roriguez LA, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. *Lancet* 1994; **343**: 769–772.
2 Boyd CM, Darer J, Boult C, Fried LP, Boult L, Wu AW. Clinical practice guidelines and quality of care for older patients with multiple comorbid diseases: implications for pay for performance. *JAMA* 2005; **294**: 716–724.
3 Man-Son-Hing M, Laupacis A. Anticoagulant-related bleeding in older persons with atrial fibrillation: physicians' fears often unfounded. *Arch Intern Med* 2003; **163**: 1580–1586.
4 Schmader KE, Hanlon JT, Pieper CF *et al.* Effects of geriatric evaluation and management on adverse drug reactions and suboptimal prescribing in the frail elderly. *Am J Med* 2004; **116**: 394–401.
5 Kalyanasundaram A, Lincoff AM. Managing adverse effects and drug-drug interactions of antiplatelet agents. *Nat Rev Cardiol* 2011; **8**: 592–600.

Pituitary insufficiency: A cause of hypoglycemia in an elderly diabetic patient

Dear Editor,

Hypoglycemia most likely occurs in the elderly as a result of poor glucose tolerance. The most common cause of hypoglycemia in elderly patients is antidiabetic drugs. Adrenal insufficiency, insulinoma and pituitary insufficiency are rare causes of hypoglycemia in older age.[1] Particularly in old patients, non-specific findings, such as weakness, fatigue and loss of appetite caused by pituitary insufficiency, might be attributed to aging.[2] Here, we reported an elderly patient with diabetes mellitus and hypopituitarism, presenting with refractory hypoglycemia and acute renal failure under therapy with oral antidiabetic drugs.

A 67-year-old woman was referred to geriatric clinic with symptoms of confusion, irritability, slowness of speech and movements, loss of appetite, nausea, and vomiting. A physical examination of her vital signs showed blood pressure 80/50 mmHg, pulse rate 104/min, body temperature 37.7°C and respiration 24/min. The patient was lethargic with incomplete cooperation (Karnofsky performance score of 30%). She had been taking metformin 2000 mg/day and gliclazide 30 mg/day with the diagnosis of diabetes for 2 years. In the biochemical examination, blood glucose, blood urea-nitrogen, creatinine, sodium and potassium were 32 mg/dL, 60 mg/dL, 3.2 mg/dL, 132 mmol/L and 4.9 mmol/L, respectively. After she was admitted to the geriatric clinic, her glucose infusion was given. Our initial evaluation of the clinical and laboratory parameters suggested that it could be acute renal failure as a result of dehydration and hypoglycaemia, which were the consequence of the prolonged effect of gliclazide. For this reason, oral antidiabetic drugs were discontinued, and glucose infusion was carried out. During her clinical follow up, we realized that her kidney functions had substantially increased. However, hypoglycemia persisted. Afterwards, all of the persistent hypoglycemia, hyponatremia and hypotension were evaluated, and the results were considered to be hypocortisolemia. The patient's other laboratory results, which were obtained during a hypoglycemia period, are presented in the Table 1. The basal serum cortisol (1.38 μg/dL) and adrenocorticototropic hormone levels (less than 0.3 U/L) showed strong evidence of cortisol deficiency. Due to these results, pituitary insufficiency was diagnosed. However, magnetic resonance imaging and magnetic resonance angiography did not show any structural or vascular abnormalities in the hypophysis and brain. Once prednisolone (7.5 mg/day) treatment

Table 1 Endocrinological laboratory results

Parameters		Normal range
Blood cortisol	1.38 ug/dL	6.2–19.4 ug/dL
TSH	0.055 uIU/mL	0.4–4.2 uIU/mL
Free T4	13.24 pmol/L	10.3–23.2 pmol/L
IGF-1	1.00 mg/L	1.73–5.11 mg/L
GH	<3 μg/L	
PRL	0.57 ng/mL	3–20 ng/mL
FSH	2.02 mIU/mL	25.8–134.8 mIU/mL
LH	1.36 mIU/mL	7.7–58.5 mIU/mL
Estradiol	27.96 pg/mL	5–54.7 pg/mL
C peptide	1.02 ng/mL	0.9–7.1 ng/mL
Insuline	2.83 μU/mL	3–28 μU/mL

All the laboratory results were measured between 08.00 hours and 09.00 hours, and confirmed by a second determination.
FSH, follicule stimulating hormone; GH, growth hormone; IGF1, insulin-like growth factor-1; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid stimulating hormone; T4, thyroxine.

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γ-Secretase Modulators and Presenilin 1 Mutants Act Differently on Presenilin/γ-Secretase Function to Cleave Aβ42 and Aβ43

Masayasu Okochi,[1,*] Shinji Tagami,[1] Kanta Yanagida,[1] Mako Takami,[2,3] Takashi S. Kodama,[1] Kohji Mori,[1] Taisuke Nakayama,[1] Yasuo Ihara,[2,3] and Masatoshi Takeda[1]

[1]Neuropsychiatry and Neurochemistry, Department of Integrated Medicine, Division of Internal Medicine, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan

[2]Department of Neuropathology, Faculty of Life and Medical Sciences, Doshisha University, Kizugawa 619-0225, Japan

[3]JST, CREST, Kawaguchi, 332-0012, Japan

*Correspondence: mokochi@psy.med.osaka-u.ac.jp

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SUMMARY

Deciphering the mechanism by which the relative Aβ42(43) to total Aβ ratio is regulated is central to understanding Alzheimer disease (AD) etiology; however, the mechanisms underlying changes in the Aβ42(43) ratio caused by familial mutations and γ-secretase modulators (GSMs) are unclear. Here, we show in vitro and in living cells that presenilin (PS)/γ-secretase cleaves Aβ42 into Aβ38, and Aβ43 into Aβ40 or Aβ38. Approximately 40% of Aβ38 is derived from Aβ43. Aβ42(43) cleavage is involved in the regulation of the Aβ42(43) ratio in living cells. GSMs increase the cleavage of PS/γ-secretase-bound Aβ42 (increase k_{cat}) and slow its dissociation from the enzyme (decrease k_b), whereas PS1 mutants and inverse GSMs show the opposite effects. Therefore, we suggest a concept to describe the Aβ42(43) production process and propose how GSMs act, and we suggest that a loss of PS/γ-secretase function to cleave Aβ42(43) may initiate AD and might represent a therapeutic target.

INTRODUCTION

Alzheimer disease (AD) amyloid-β42 peptide (Aβ42) and Aβ43 are generated from β-amyloid protein precursor (βAPP) and accumulate in senile plaques (Gravina et al., 1995). Because Aβ is secreted as multiple peptide species with different C termini, intramembrane proteolysis of Aβ by presenilin (PS)/γ-secretase (De Strooper et al., 1998, 1999; Sherrington et al., 1995; Struhl and Greenwald, 1999; Wolfe et al., 1999) does not occur at a unique site. However, because even small elevations in the ratio of Aβ42(43) to total Aβ (Aβ42[43] ratio) in secreted Aβ by PS or βAPP mutations trigger familial AD (Kuperstein et al., 2010; Scheuner et al., 1996; Suzuki et al., 1994), the proteolysis is regulated strictly in this aspect. How the variation in Aβ is generated remains unclear.

How βAPP-CTF is cleaved into Aβ42 is controversial because of conflicting findings. First, cleavage at the ε-site generates primarily long fragments, namely Aβ48 and Aβ49, and is followed by stepwise cleavage of every three amino acid residues starting at the C terminus (Qi-Takahara et al., 2005). Two distinct lines have been proposed for Aβ40 and Aβ42 production. Second, cleavage at the γ-site does not correlate directly with cleavage at the ε-site (He et al., 2010).

The ratio of APL1β28, a surrogate marker of Aβ42, to total APL1β is elevated in the cerebrospinal fluid of patients with sporadic AD, including those in the mild cognitive impairment stage and in those with familial AD (Yanagida et al., 2009). Thus, an increase in the Aβ42 ratio in the brain may play a role in the etiology of most AD cases, and the mechanism underlying the regulation of the Aβ42 ratio is a central issue in understanding AD.

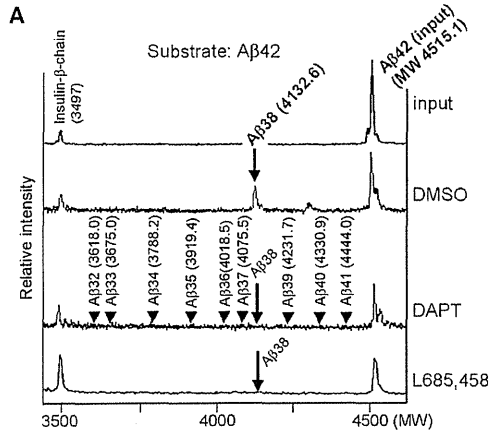
γ-Secretase modulators (GSMs) are disease-modifying drugs that specifically reduce Aβ42 generation (Kounnas et al., 2010; Weggen et al., 2001); some GSMs are being studied in clinical trials. Despite the development of GSMs (Wolfe, 2012), their mechanism of action (Chávez-Gutiérrez et al., 2012) remains unclear.

Given that the VVIA peptide located between the γ38 and γ42 cleavage sites was detected in an in vitro βAPP-CTF cleavage assay (Takami et al., 2009), we asked whether Aβ42(43), formerly considered a product of PS/γ-secretase, could also be a substrate for PS/γ-secretase. Here, we show that Aβ42(43) is an intermediate of PS/γ-secretase, a finding that provides important insight into the regulation of the Aβ42(43) ratio. Our results will help elucidate the mechanism underlying the actions of GSMs and PS mutants.

RESULTS

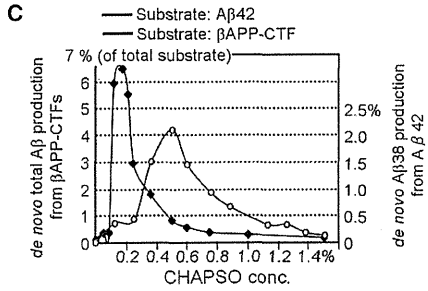
AD-Associated Aβ42 Is a Substrate of PS/γ-Secretase

Initially, we performed a modified in vitro γ-secretase assay in which we used CHAPSO (3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxypropanesulfonate) at its critical micelle concentration of 0.5% instead of 0.25%, the concentration used in the original method (Li et al., 2000). We experimented with using Aβ42 as a substrate instead of the βAPP-C-terminal fragment (βAPP-CTF) (Chávez-Gutiérrez et al., 2012). Surprisingly, Aβ38 was generated de novo from Aβ42 by PS/γ-secretase, a process that we refer to as "Aβ42 cleavage" (Figure 1). MALDI-TOF mass



B Substrate: Aβ42 (1 pmol)

	VVIA	GGVV	GVV	VVI	VIA (fmol)
0 min	1.1	0	0	0	0
10 min	18.1	0	0	0	0.4
20 min	37.4	0	0	0	1.1
30 min	52.5	0	0	0	1.4
L685,458 (30 min)	0	0	0	0	0



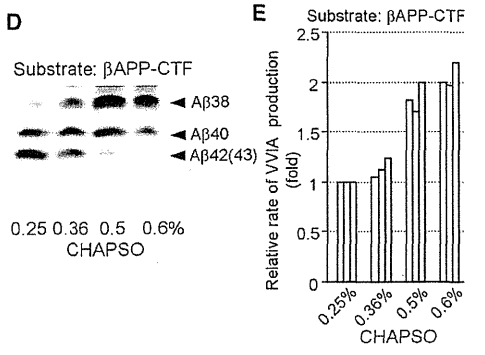


Figure 1. Detection of Aβ and Related Small Peptide Species

Aβ42 and βAPP-CTF cleavage assays were performed at 37°C for 30 min.

(A) Immunoprecipitation-MS analysis of products from the Aβ42 cleavage assay.

(B) Small Aβ-derived peptides (Takami et al., 2009) detected in the Aβ42 cleavage assay.

(C) Levels of total Aβ from βAPP-CTF (black line) and Aβ38 generated from Aβ42 (red line) at various CHAPSO concentrations (0%–1.5%).

(D) Immunoprecipitation-immunoblot detection of Aβ. The volume of each reaction for immunoprecipitation was adjusted to contain the same amount of total Aβ.

(E) Relative rate of the VVIA produced in (D), as defined by [VVIA]/[TVI]. The actual individual data from each of the three experiments are plotted.

See also Figures S1, S2, and S4.

spectroscopy (MS) showed a de novo product of 4132.6 Da, which matches ionized Aβ38 (Figure 1A). Liquid-chromatography tandem MS (LC-MS/MS) (Takami et al., 2009) showed high levels of VVIA tetrapeptide between Aβ38 and Aβ42 (Figure 1B). No further cleavage of Aβ38 was observed, indicating that Aβ38 was the final product (Figure S1A). We also observed a small amount of VIA (~1/40th the amount of VVIA), the tripeptide between Aβ39 and Aβ42, indicating very low-level production of Aβ39 (Figure 1B). Cleavage of Aβ42 was eliminated by γ-secretase inhibitors (Figures 1A and 1B). We also found that the N terminus of the substrate Aβ42 was not necessarily the first residue, because Aβ11–42 was also cleaved (Figure S1B). Collectively, these results indicate that Aβ42 is cleaved into Aβ38 and Aβ39. Irrespective of how cleavage at the γ42-site occurs (Qi-Takahara et al., 2005; He et al., 2010), our current results demonstrate that Aβ42, the pathological PS/γ-secretase product, is also a substrate for PS/γ-secretase.

We next studied Aβ42 cleavage, when βAPP-CTF is the substrate. Cleavage of Aβ42 in the new assay was optimal at ~0.5% CHAPSO, whereas cleavage of βAPP-CTF in the conventional assay was optimal at ~0.2% CHAPSO (Li et al., 2000) (Figure 1C). Given this result, we considered the possibility that the Aβ42 ratio in the de novo Aβ generation in the βAPP-CTF cleavage assay might decrease at a higher CHAPSO concentration (~0.5%). As the CHAPSO concentration increased, the relative ratio of Aβ42(43) production decreased and the ratio of Aβ38 production increased (Figure 1D; Figure S2A). Surprisingly, the relative ratio of Aβ42(43) to Aβ40 was ~0.1 at ~0.5% CHAPSO. Thus, we suspected that the much higher Aβ42(43) ratio produced in the conventional βAPP-CTF cleavage assay than that secreted from living cells may be because the assay was performed in the presence of ~0.25% CHAPSO. In this regard, the in vitro γ-secretase assay in the presence of ~0.5% CHAPSO may be a better model.

Because Aβ42 levels are determined by the balance between its rate of production and its rate of degradation, we measured further the levels of small peptides produced by βAPP-CTF cleavage (Figure 1E). The relative amount of VVIA produced by βAPP-CTF cleavage increased as the Aβ38 ratio increased. These data suggest that the relative levels of Aβ42 are also a function of Aβ42 cleavage. Moreover, CTFγ38/40/42/43, the counterparts of direct cleavage at the Aβ38/40/42/43 sites, have never been observed (Gu et al., 2001). Collectively, our results indicate that Aβ42 is an intermediate stopover product of the cleavage of βAPP-CTF by PS/γ-secretase.

GSMs and Mutant PS1/γ-Secretases Increase and Decrease Aβ42 Cleavage in Living Cells, Respectively

Next, we asked whether the Aβ42 cleavage process plays a role in conditions where the Aβ42 ratio in secreted Aβ changes in vitro and in living cells. First, we examined whether GSMs and inverse GSMs (iGSMs) (Kukar et al., 2005) change the rate of Aβ42 cleavage in vitro when the enzyme activity is low (0.25% CHAPSO) or high (0.5% CHAPSO). Strikingly, in the presence of 0.25% CHAPSO, all GSMs tested (GSM1, Eisai, Compound W, and Sulindac sulfide) increased the relative rate of Aβ42 cleavage (Figure 2A; Table S1A). In contrast, in the presence of 0.5% CHAPSO, the iGSMs tested (fenofibrate and

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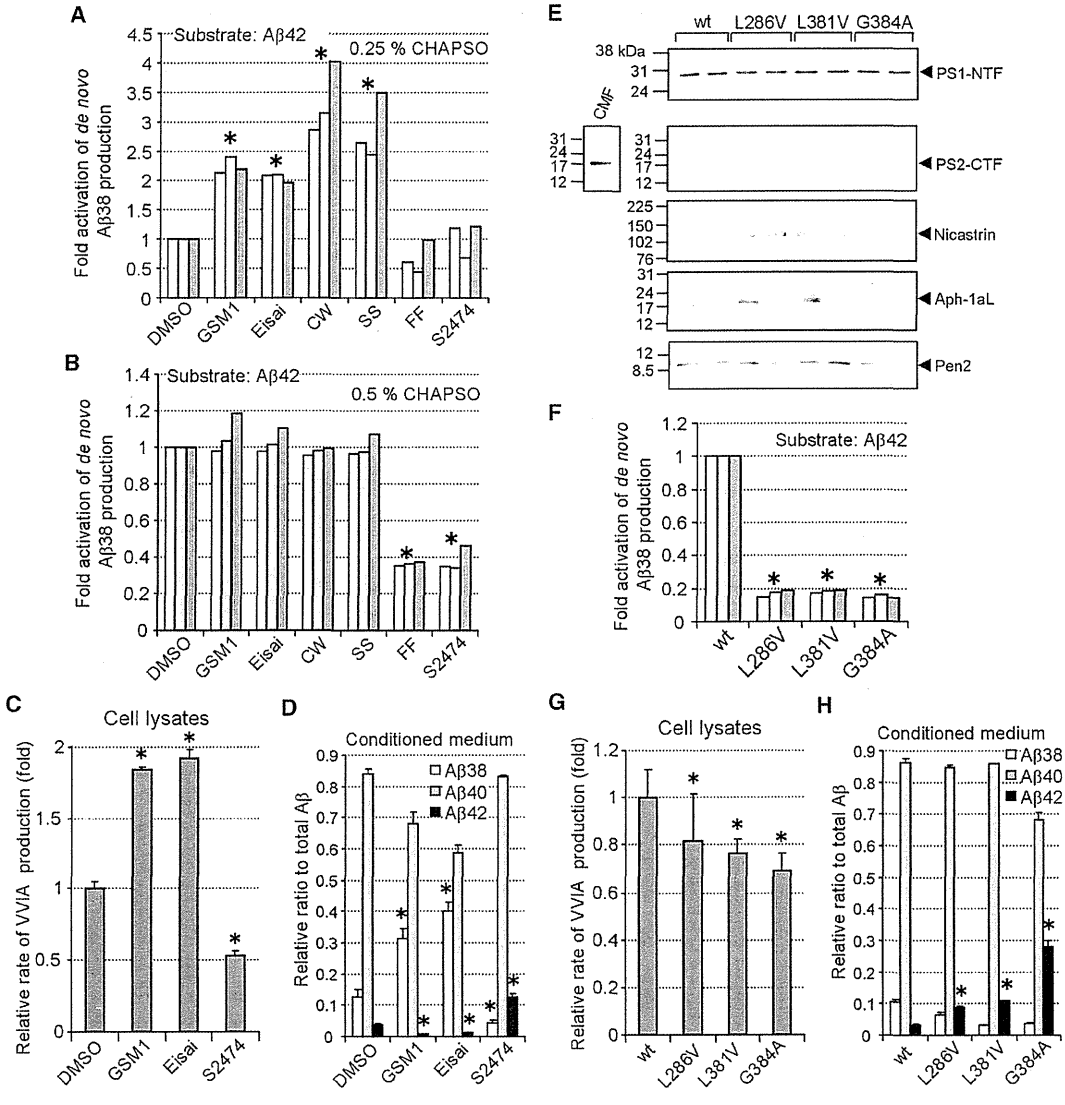

Figure 2. Effects of GSMs/iGSMs and Mutant PS1/γ-Secretases on Aβ42 Cleavage

(A) Fold activation of Aβ42 cleavage by GSMs/iGSMs in the presence of 0.25% CHAPSO. We extracted the PS/γ-secretase fraction from HEK cells stably expressing WT PS1 (Figure S2B). A total of 40 μM GSM1, 10 μM Eisai, 10 μM compound-W (CW), 10 μM SS, 50 μM fenofibrate (FF), or 10 μM S2474 was added to the in vitro assay.

(B) Fold activation of Aβ42 cleavage by GSMs/iGSMs in the presence of 0.5% CHAPSO.

(C) Fold changes of the relative VVIA levels in cell lysates treated with GSMs/iGSM. A total of 4 μM GSM1, 1 μM Eisai, or 30 μM S2474 was added to the cultured medium.

(D) The relative ratio of Aβ species in conditioned medium. The levels of Aβ38, Aβ40, and Aβ42 were measured by ELISA.

(E) Immunoblotting of purified PS/γ-secretase fractions. To show that the same level of each mutant PS1/γ-secretase was used in each reaction, we immunoblotted each fraction with antibodies against all four indispensable elements of PS1/γ-secretase: PS1/2, nicastrin, APh-1-a, and PEN-2. We detected almost equal band densities for all four proteins of mutants and WT PS1/γ-secretase fractions. Exogenous PS1 derivatives displaced the endogenous WT PS2 in the PS/γ-secretase complex. Note that a certain mutant contained a higher level of nicastrin, and thus was omitted from the analysis.

(F) Fold activation of Aβ42 cleavage by purified mutant PS1/γ-secretase.

(G) Fold changes of the relative VVIA levels in cell lysates stably expressing PS1 mutants.

(H) The relative ratio of Aβ species in conditioned medium.

The Aβ38 level was measured by ELISA. Asterisks indicate $p < 0.05$, Welch's t test. Error bars represent SD. The actual individual data from each of the three experiments are plotted in (A), (B), and (F). See also Figures S1 and S2.

S2474) decreased the relative rate of Aβ42 cleavage (Figure 2B; Table S1B).

We do not know how CHAPSO affects the rate of Aβ42 cleavage in vitro. However, in the presence of 0.25% CHAPSO, when Aβ42 cleavage activity is low, iGSMs did not slow the reaction (Figure 2A). Similarly, GSMs did not increase Aβ42 cleavage in the presence of 0.5% CHAPSO, when the activity is high (Figure 2B). These findings suggest that the effects of CHAPSO and GSMs/iGSMs are related.

Next, we investigated whether an increase in Aβ42 cleavage by GSMs is responsible for the decrease in the Aβ42 ratio in living cells. We added GSMs and iGSMs to cultured HEK cells expressing wild-type (WT) and Swedish mutant (sw) βAPP stably, which were the same cell lines used for extracting PS1/γ-secretase for the in vitro experiments. We immediately boiled the treated cells for 2 min to inhibit completely the active degradation of tri-, tetra-, and pentapeptides in the living cells. We extracted the soluble fraction from the resultant cell lysate and measured the levels of the small peptides by LC-MS/MS (Figures 2C and 2G).

Hereafter, the mention of an increase or a decrease in the levels of each peptide associated with the generation of secreted forms of Aβ (e.g., VVIA, VVIAT, and IAT) implies that the relative ratio of a peptide was calculated in relation to that of the sum of peptides associated with Aβ generation (i.e., ITL, VIT, VIV, TVI, IAT, VVIA, and VVIAT) (Figures 2, 3, and 4). GSM-treated cells, which secreted a lower ratio of Aβ42 (Figure 2D; Table S1D), contained a higher ratio of VVIA (produced by cleavage of Aβ42 into Aβ38) than did DMSO-treated cells (Figure 2C; Table S1C). In contrast, iGSM-treated cells, which secreted a higher ratio of Aβ42 (Figure 2D), contained a lower ratio of VVIA (Figure 2C). These data indicate that GSMs and iGSMs increase and decrease, respectively, the rate of Aβ42 cleavage in living cells.

Next, because the Aβ42 ratio in secreted Aβ is increased in most PS1 pathological mutants (Scheuner et al., 1996), we examined whether PS1 mutants integrated into the PS/γ-secretase complex exhibit a reduced rate of Aβ42 cleavage. We extracted the PS/γ-secretase fraction (Figure 2E; see also Figures S2F and S2B–S2D) (Winkler et al., 2009) from HEK cells stably expressing WT or mutant (L286V, L381V, or G384A) PS1 (Figures S2E and S2F). The same amount of enzyme complex was included in each reaction (Figure 2E). We found that the rate of Aβ42 cleavage was lower for mutant than for WT PS1/γ-secretase (Figure 2F; Table S1E), which is consistent with a previous report (Chávez-Gutiérrez et al., 2012). The results are reminiscent of the fact that cells expressing mutant PS1/γ-secretases generally secrete less total Aβ than do WT cells (Shen and Kelleher, 2007). However, it is of note that the reduced rate of Aβ42 cleavage described here is different from the overall loss of function of PS/γ-secretase.

We investigated whether reducing Aβ42 cleavage by mutant PS/γ-secretases could increase the Aβ42 ratio in living cells. We cultured WT or mutant PS1-expressing cells coexpressing sw βAPP (the same cell line used for the in vitro experiments) and analyzed the cell lysates by LC-MS/MS. The relative levels of VVIA were lower in the lysates of mutant cells than in WT cells (Figure 2G; Table S1F). We confirmed the increased Aβ42 ratio in the conditioned medium of the mutant-expressing cells (Figure 2H; Table S1F). These data demonstrate that mutant PS1/γ-secretases decrease the rate of Aβ42 cleavage in living cells. Collectively, our data suggest that the Aβ42 cleavage process is associated with the Aβ42 ratio in secreted Aβ.

A New Concept for the Production of Bona Fide Aβ42

To gain insight into the regulation of Aβ42 cleavage, we next examined how GSMs, iGSMs, and mutant PS1/γ-secretases alter Aβ42 cleavage activity in vitro. The conversion of Aβ42 into Aβ38 can be described by the following scheme and with the following rate constants:

$$[PS/\gamma - secretase] + [A\beta 42] \underset{k_b}{\overset{k_a}{\rightleftharpoons}} [PS/\gamma - secretase/A\beta 42 - complex] \xrightarrow{k_{cat}} [A\beta 38] + [PS/\gamma - secretase]$$

This equation can be applied to the production of "free Aβ42" from de novo Aβ42 (shown in the diagram in Figure 3A). Escape from further cleavage and production of free Aβ42 both require that the de novo-generated bound Aβ42 dissociates from PS/γ-secretase. We suggest this concept to explain the production of bona fide Aβ42. According to the model, k_{cat} (unimolecular rate constants) and k_b (dissociation rate constants) values would be relevant to the generation of free Aβ42.

One may think that once βAPP-CTF and PS/γ-secretase form a complex, intermediate long Aβ does not dissociate from the enzyme during the stepwise cleavages. According to the model, the k_{cat} values of the Aβ42 cleavage should vary depending on the substrate. However, without any changes in the relative position of long Aβ species to PS/γ-secretase, the stepwise cleavages might be interrupted. Moreover, we showed clearly that Aβ42, Aβ43, Aβ45, and Aβ46 can bind to PS/γ-secretase and undergo cleavage (Figures 1 and 4). It is unknown whether all of the intracellular Aβ45 and Aβ46 (Qi-Takahara et al., 2005) bind to PS/γ-secretase. Based on these findings, we suggest that the long Aβ species undergoes association/dissociation events with PS/γ-secretase. Therefore, we revised the formulas describing the stepwise cleavage process proposed originally by Takami et al. (2009) (Figure 3B). We introduced the association/dissociation steps clearly for each cleavage step and did not consider that the cleavage at every three amino acid residues is an essential part of the cleavage process. Aβ42 and Aβ43 correspond to $Aβ_{Xn}$ in the revised scheme. Aβ45 and Aβ46 correspond to $Aβ_{X(n-1)}$. Whether the various free $Aβ_{Xn}$ products produced in each step remain at the membrane depends on their physicochemical nature. According to our model, the k_{cat} values of the Aβ42 cleavage should be the same, regardless of the substrate (e.g., βAPP-CTF and Aβ42).

GSMs and Mutant PS1 Increase and Decrease, Respectively, the Velocity at which Bound Aβ42 Is Cleaved to Aβ38 In Vitro

First, to obtain the relative k_{cat} values for Aβ42 cleavage, we performed enzyme kinetic analysis of Aβ42 cleavage over a range of Aβ42 concentrations (Figures 3C–3E; see also Tables S2A–S2C). The Aβ42 cleavage reaction conformed to Michaelis-

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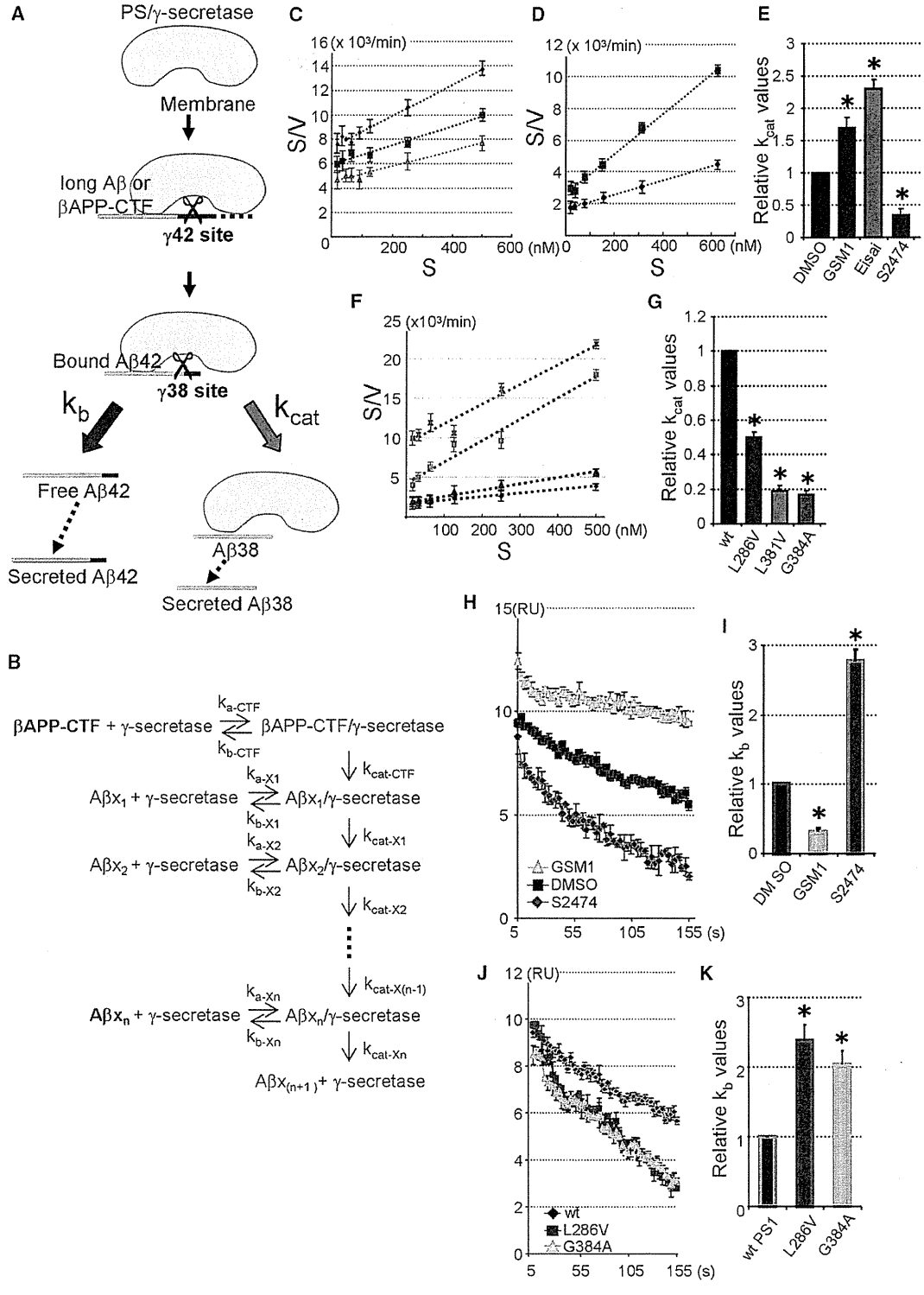

Figure 3. Enzyme Kinetic Analysis of Aβ42 Cleavage and Biacore Analysis of Aβ42 Dissociation from PS1/γ-Secretase

(A) Proposed reaction mechanism for Aβ42 production.

(B) Proposed formulas of stepwise cleavage for Aβ production.

(C) Hanes-Woolf plots of the Aβ42 cleavage in the presence of 0.25% CHAPSO and GSMs (DMSO, black diamonds; GSM1, green squares; Eisai, blue triangles).

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Menten kinetics (Figures S3A–S3D). Next, we examined whether GSM or iGSM alters the k_{cat} for Aβ42 cleavage. Hanes-Woolf plots were used to determine the V_{max} values (Figures 3C, 3D, and 3F). Because Aβ42 was in excess in the reactions, we assumed that $V_{max} = k_{cat}$ [PS/γ-secretase]. The relative k_{cat} value was larger in the presence of GSM1 or Eisai than with WT PS/γ-secretase alone, whereas the value was smaller in the presence of S2474 than with WT PS/γ-secretase (Figure 3E; Table S2C). Depending on CHAPSO concentration (0.25% or 0.5%), the y intercepts of the Hanes-Woolf plots differed, indicating that CHAPSO noncompetitively modified the action of PS/γ-secretase. We next performed reactions using equal amounts of each mutant and WT PS1/γ-secretase (Figure 3F; Table S2D). The relative k_{cat} values were smaller for PS1 L286V, L381V, and G384A/γ-secretase than for WT PS/γ-secretase (Figure 3G; Table S2E). Thus, in the assay conditions used, GSMs increased the velocity at which bound Aβ42 was cleaved to Aβ38, whereas both PS1 mutants and iGSM reduced the velocity of the cleavage.

A GSM Reduces the Velocity at which Bound Aβ42 Dissociates from WT PS1/γ-Secretase, but the Complex of Mutant PS1/γ-Secretases and Aβ42 Dissociates Faster

We also used Biacore binding analysis to measure the relative dissociation rate constant k_b for the complex of Aβ42 bound to PS1/γ-secretase. Aβ42 was immobilized to the sensor tip, and purified PS1/γ-secretases were injected as the analytes. We tried to measure the k_b values to show how fast Aβ42 dissociates from the active center of PS/γ-secretase. We performed the Biacore assay with PS/γ-secretase preincubated in the presence or absence of L685,458, a transition state mimic that blocks the active site of PS/γ-secretase (see Figures S3E–S3J). We assumed that subtracting the resonance unit (RU) value for the L685,458–PS/γ-secretase complex binding to Aβ42 from the RU value for PS/γ-secretase alone (without mixing with L685,458) binding to Aβ42 would give the RU value for PS/γ-secretase, which holds Aβ42 in its active center (see Figures 3H and 3J). Using the RU values, we calculated k_b values for the dissociation of the bound Aβ42 from the active center.

We studied whether GSM1 or S2474 affects the k_b value. During the period from 5 s to 155 s in the dissociation phase, the washout curves of compounds tested with Aβ42 showed simple one-step dissociation with the single exponential rate expected from the model (Figure 3H; Table S2F). The relative k_b values of dissociation in the presence of GSM1 and S2474 were smaller and larger, respectively, than the value for WT PS/γ-secretase alone (Figure 3I; Table S2G). The results indicate that GSM1 and S2474 decreased and increased the rate of dissociation of Aβ42 from the active center of PS/γ-secretase by 0.36 and 2.7 times, respectively, compared with the DMSO control in the assay condition. We also performed similar experiments to measure the relative k_b values for the complex of Aβ42 with WT and mutant PS1/γ-secretase (Figure 3J; Table S2H). The dissociation rates of Aβ42 from L286V and G384A mutant PS1/γ-secretases were 2.4 and 2.0 times larger, respectively, than the rate for WT PS1/γ-secretase (Figure 3K; Table S2I). These data suggest that the velocity at which bound Aβ42 dissociates from PS1/γ-secretase contributes to the changes in the Aβ42 ratio in secreted Aβ caused by the compounds and some mutants. However, we are not yet able to show the extent of the relative effects of the two factors (i.e., k_{cat} and k_b) when the Aβ42 ratio changes in living cells.

Aβ43, Another Long Aβ Species, Is Cleaved into Aβ40 or Aβ38 by PS/γ-Secretase in Living Cells

Aβ43 is another long species of Aβ (Saito et al., 2011). We also investigated whether Aβ43 undergoes further proteolysis in a manner similar to Aβ42. MALDI-TOF MS (Figure 4A) and LC-MS/MS (Figure 4B) showed that Aβ43 was cleaved to Aβ40 or Aβ38 by PS/γ-secretase in vitro. Thus, Aβ38 has multiple precursors. This was confirmed by the in vitro βAPP-CTF cleavage assay (Figure 4C). Why PS/γ-secretase cleaved the substrate at only one of two sites remains unclear. The production of Aβ37 and GVV indicates minor but further cleavage of de novo Aβ40 (Figures 4A and 4B; Figures S1C and S1D).

We measured the amounts of tri-, tetra-, and pentapeptides produced during the stepwise processing of βAPP in living cells (Figure 4D; Figure S4A). Approximately 40% of Aβ38 was derived from Aβ43 (Table S3A). GSMs increased the relative rate of Aβ43 cleavage into Aβ38 (i.e., the level of VVIAT relative to that of total Aβ-related small peptides) (Figure 4E; Table S3B), whereas iGSM and mutant PS1/γ-secretases decreased the rate (Figure 4E and 4F; Table S3C). These results are very similar to the effects of the compounds and the mutants on Aβ42 cleavage (see Figures 2C and 2G; summarized in Figures 4I and 4J). However, both the tested GSM/iGSM (Figure 4G; Table S3D) and some mutant PS1/γ-secretases (i.e., PS1 L381V and G384A) (Figure 4H; Table S3E) decreased the rate of Aβ43 cleavage into Aβ40 (i.e., IAT). Thus, the cleavage of Aβ43 into Aβ38 and that into Aβ40 were affected differently by GSMs.

Next, we asked whether the relative level of Aβ38 derived from Aβ42 and that derived from Aβ43 (i.e., VVIA and VVIAT) in living

(D) Hanes-Woolf plots of Aβ42 cleavage in the presence of 0.5% CHAPSO and iGSM (DMSO, black diamonds; S2474, red squares).
(E) The relative k_{cat} values (n = 4) of Aβ42 cleavage in the presence of GSMs/iGSM.
(F) Hanes-Woolf plots of Aβ42 cleavage by WT and mutant PS1/γ-secretase (WT PS1, black diamonds; PS1 L286V, red triangles; PS1 L381V, blue squares; PS1 G384A, purple crosses) in the presence of 0.5% CHAPSO.
(G) The relative k_{cat} values of Aβ42 cleavage by WT and mutant PS1/γ-secretase.
(H) Fitted curves of dissociation for DMSO (purple/black), GSM1 (18 μM, green/gray), and S2474 (135 μM, blue/red).
(I) k_b values (n = 3 for each) in the presence of GSM/iGSM treatment compared with those obtained in the presence of DMSO treatment.
(J) Fitted curves of dissociation (WT PS1/γ-secretase, blue/black; PS1 L286V/γ-secretase, purple/red; PS1 G384A/γ-secretase, green/gray).
(K) k_b values of mutant PS1/γ-secretases relative to those of WT PS1/γ-secretase.
Asterisks indicate $p < 0.05$, Welch's t test. Error bars represent SD. See also Figure S3.

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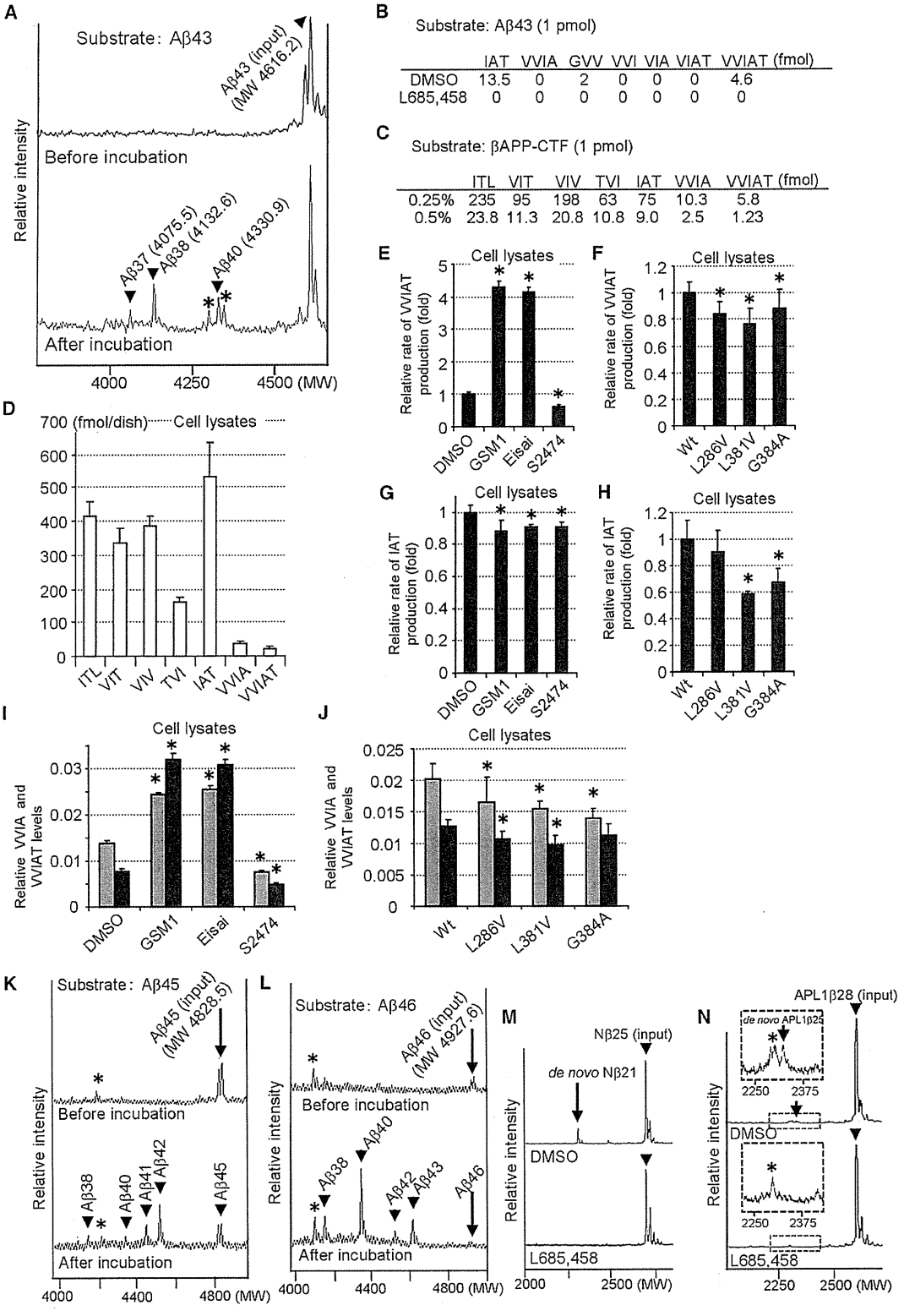

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