For accurate measurement, the adoption of a generic QOL measure, in addition to a specific symptom measure, is recommended for future studies.

Estimated Mechanism of MCI Development

Toxicity of Chemotherapy

One of the major hypotheses explaining the development of MCI is that it is a direct effect of chemotherapeutic neurotoxicity. Drugs which are a standard part of the chemotherapy regimen for breast cancer, such as methotrexate, paclitaxel and 5-FU, are recognized as neurotoxic[18]. In particular, long-term utilization of these agents often leads to neurological dysfunction. Methotrexate reduces the metabolic functioning of the brain inducing fatigue, somnolence and seizures, and is most frequently associated with late symptoms[6]. 5-FU is known to directly damage the Purkinje cells, causing dysphasia and dyskinesia resulting in acute cerebellar syndrome[19]. Recent chemotherapy regimens include docetaxel and paclitaxel, which are also considered to cause damage to the neurological system, specifically the optic nerve and sensory functions[20, 21]. Evidence that further supports the hypothesis that MCI is a direct effect of chemotherapy is found in van Dam's study which reported a dose related effect[22].

Hormone Related Changes Reducing Estrogen Levels

Reproductive hormonal level changes, in particular, the fact that estrogen or androgenic precursors levels decline following chemotherapy, is speculated to cause MCI on the grounds of frequent ovarian failure[3, 23]. In short, the chemotherapy regimen is considered to activate cholinergic action and changes in neural transmitters, causing MCI symptoms such as memory loss. In fact, memory loss caused by naturally induced menopause and dementia is often treated by hormone replacement therapy[24, 25], and the positive effect of estrogen on the verbal memory of breast cancer patients has been scientifically proven in a controlled study[26].

While MCI has been commonly observed among female breast cancer patients, it is important to observe patients with other types of cancer, or male patients receiving chemotherapy. Interestingly, no disorder was observed among mixed small cell lung cancer patients of both sexes, according to a trial that divided participants into treated and untreated groups[27]. The same author found a high frequency of neurobehavioral abnormalities in mixed-site cancer patients (including breast cancer) treated with difluorodeoxycytidine (dFdC), but concluded that this possibly resulted from the use of interferon[7]. Prostate cancer patients receiving androgen suppression monotherapy seemed to suffer from MCI[28], and an association between cognitive deficits and hyperthyroidism was observed[29]. Moreover, the fact that chemotherapy-induced symptoms occur more frequently among pre-menopausal women, supposing chemical castration, reinforces the role of hormones on cognitive function. In a study of 36 pituitary tumor patients (13 males and 23 females), the author concluded that cognitive impairment may be partly because of hormonal imbalance resulting from pituitary surgery, as opposed to being caused by depression, anxiety or radiotherapy[30]. These mixed results may reflect the fact that symptoms are pluri-causal, pointing to the need to conduct well-designed studies with a proper hypothesis and methodology before we come to a conclusion.

Confounding Factors for MCI

Depression and Fatigue

Some researchers take a more cautious stance on the proposed relationship between MCI and chemotherapy. These researchers point out, for example, the fact that depression after disease diagnosis is common, insisting that this disturbed psychological status causes memory impairment or other problems. Tchen observed depression among breast cancer patients generally in her study[16], and another study found that fatigue was also significantly related to cognitive function[31]. One typical example is the research of Meyers and Cimprich which detected MCI among breast cancer patients even "prior to" the treatment which persisted over time[27, 32]. They thought that the cause was severe depression, fatigue, insomnia and loss of appetite because of the diagnosis of cancer.

Age Related Factors

Many of the women diagnosed with breast cancer are peri-menopausal. During this time, due to fluctuating estrogenal levels, women tend to expe-

rience various functional problems. Physically, hot flushes, cystitis, and irregular bleeding occur commonly, while mentally, memory loss, mood disorder and low sexual interest are often reported. In addition to these physical changes, Bender estimates that general changes in women's familial situations, such as children leaving home can also negatively affect women's mental status[3].

Tamoxifen Use

Unless patients are both ER and PR negative, hormonal therapy using tamoxifen along with chemotherapy is a standard adjuvant treatment in most medical facilities in developed countries. As mentioned earlier, hormonal therapy using tamoxifen, an estradiol antagonist, is thought to block the estradiol receptor, affecting the serotonergic mechanism[33]. Several researchers have proven the anti-estrogenic effect of tamoxifen following cerebral metabolism disturbance[24, 34]. In particular, Paganini-Hill found that more women who had used tamoxifen for around 5 years (48-71 months) reported memory problems than non-users[35]. It is necessary to conduct clinical research with a patient group being treated solely with chemotherapy, because the employment of a research design that controls for tamoxifen use is an indispensable step for future studies.

Other Confounding Factors

Harder reported in his research on bone marrow transplantation that cognitive function is largely dependent on the patient's general health status and education level, with fatigue playing an additional role[36]. Cognitive failure related to the use of narcotics to control cancer pain has also been reported[37]. Other studies suggest many confounding factors such as infection, fever, medication, nutrition deficiency and sleep disorders, implicated problematically between cognitive function and chemotherapy[3, 6]. The impacts of such confounding factors are more than negligible, thus controlling for them in future studies is necessary.

Methodology to Measure MCI

The difficulty in studying chemotherapy-induced MCI is compounded by the lack of methodological consistency and difficulty in measuring tenuous symptoms. Currently, the predominant three methods of evaluating the effects of chemotherapy on cognitive function are: neuro-psychological tests, self-rated subjective questionnaires and physiological methods.

Objective Neuro-Psychological Tests

Neuro-psychological methods are often based on questionnaires or practical tests. Most of the studies reviewed in this article employed a battery of specific cognitive function tests, such as trail making, complex figure drawing, digit span, and the Wechsler Memory Scale verbal memory test (WMS)[38] (Table 1). Detailed battery tests are able to describe adequately the multi-dimensional cognitive status of patients, however, their length risks being burdensome to respondents. An alternative test, the Wechsler Adult Intelligence Scale (WAIS) is a measurement of intellectual status in the general population and is broadly used to measure cognitive function[39]. The test's high sensitivity to subtle changes in cognitive function has been reported. Measures for dementia, such as the Mini Mental State Examination (MMSE)[40], might also be used to measure MCI in cancer patients[41]. As an advantage, it consists of only about ten questions and aims primarily to measure the patient's capacity for memory, solution finding, and spatial orientation. However, the sensitivity of these measures to detect post-treatment mild deficits in cognition may be questionable, especially among subjects who have normal cognition at baseline[42].

Functional Imaging Techniques (fMRI and PET)

As the scientific community relies increasingly on physiological or molecular biological evidence in clinical epidemiology, more researchers feel the necessity for physiological testing in MCI studies in order to measure the linking mechanisms. Functional magnetic resonance imaging (fMRI) has already been applied to dementia as a potential general screening tool[43], and used in several clinical trials to observe the long-term chemotherapy-induced brain alterations[33, 44-46]. Oncologists are frequent users of another imaging technique, ^{18}F-fluorodeoxyglucose-Positron emission tomography (FDG-PET) in order to detect remote metastasis. Limited not only to identifying malignant tissues in the body, some studies suggest that FDG-PET is sensitive enough to detect mild depression in the brain of cancer patients[47]. Similarly, in the field of geriatric and psychiatric medicine, researchers are making an attempt to apply

this technique to diagnose dementia, schizophrenia and other mental disorders[35, 48, 49]. One unpublished prospective study with MCI patients in Japan reported that researchers were able to recognize reduced blood circulation in the praecuneus and gyrus cinguli, and a study on the roles of histamine on cognition suggests the sensitivity of PET used with ^{11}C-doxepin is as high as or even higher than that of neuropsychological tests in detecting cognitive deficits due to histamine H_1 receptor antagonist[47]. PET investigation is simple, and the risk of exposure to radiation is much reduced in comparison with conventional techniques such as single photon emission tomography (SPECT). At present, more than 70 medical facilities are equipped with this apparatus in Japan, and the number is increasing. Since PET sensitively visualizes the presence of very small amounts (at pico- and nanomol levels) of neurotransmitters and receptors *in vivo*, it will be a potentially crucial tool to detect MCI-related abnormalities in neural activity and to obtain detailed information on the mechanism.

Subjective Measures

While the above measurements are used to objectively quantify cognitive status, MCI can be measured subjectively as well. For example, Berglund used a symptom list[10]. Mclachlan employed one of the most popular cancer-specific QOL measures, the EORTC QLQ-C30[31], which has two 5-point Likert scale questions on cognitive function (CF sub-scale) and includes questions such as: "Have you had difficulty in concentrating on things, like reading a newspaper or watching television?" and "Have you had difficulty remembering things?" Ahles observed consistency between subjective and objective test results[11]. In contrast, MCI is sometimes reported only subjectively by patients without any tangible signs on objective tests. For instance, Klepstad found no relationship between the score of the two questions from the QLQ-C30 questionnaire and results of the objective cognitive tests[50], a conclusion found by Schagen as well[9]. In Paganini-Hil's study, patients reported symptoms of MCI but objective measures were not able to detect overt symptoms[35]. This suggests the need to employ both subjective and objective multi-dimensional methods, preferably, for future studies.

Discussion

This study has outlined many questions relating to for chemotherapy-induced MCI, which can be addressed only by conducting an appropriately designed study. First, future research has to be randomized and longitudinal with a baseline measure point before the beginning of the chemotherapy cycle. The necessity of a such a study has been long discussed in preceding studies and reviews[3, 12, 22, 51, 52], however, no longitudinal trial with a pretreatment measure point has been published. If possible, all the confounders, such as age, education, intelligence quotient (IQ), other treatments, hormonal level, and status of menstruation need to be controlled for in the study design. At the same time, the paradoxical recruitment bias that those who show MCI are excluded from meeting the participation criteria for potential research studies has to be addressed. Setting a control group matching such confounding factors is ideal for the study. Secondly, future studies must adopt an effective and sensitive measurement method. Inconsistencies in the results of reviewed studies imply that evaluation cannot be based solely on subjective measures or objective tests. In order to determine concretely the mechanism, the use of the latest imaging technique, PET, is a potentially powerful tool. There is already a precedence for PET's use in geriatric medicine, where it is proposed that MCI be used to define the intermediate steps in the progression of dementia[53, 54]. According to the clinical definition, transient chemotherapy-induced MCI in breast cancer patients seems to be physiologically different from that defined as a predictor of dementia or Alzheimer's disease, even though there is evidence that estrogen deficiency may contribute to the onset of both cases. In applying it to MCI, PET will allow us to observe a change in cerebral activities caused by chemotherapy which cannot be judged by neuro-psychological methods only.

Knowing the possible side-effect's of treatments is essential and indispensable for medical professionals and patients. For example, while the new taxanes are considered an effective treatment option, we have an obligation to test for potential problems regarding MCI[52]. Informed consent for patients regarding these potential adverse effects, which may lead to deterioration in post treatment

QOL and changes in their ability to fulfill work and day to day life, should be supported by evidence-based knowledge. Although there may be difficulties in performing an ideal clinical trial because of high monetary and human resource costs, we have identified the need to ascertain a more comprehensive picture of symptoms and their effects on breast cancer patients. When the relationship between chemotherapy and MCI has been clarified, patients and medical professionals can more accurately define the criteria for early detection[55, 56] and consider alternative effective combinations of regimens for chemotherapy. As the benefits of using olanzapine or donepezil in supporting cognitive function have already been reported[43, 57], effective prevention, treatment, or post-treatment rehabilitation for MCI in clinical settings may be possible, using hormonal therapy, antioxidants, monoamine oxidase inhibitors, growth factors, dopamine agonists, cholinesterase inhibitors, anti-inflammatory agents, and behavioral therapy and counseling[58].

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Validation of the care notebook for measuring physical, mental and life well-being of patients with cancer

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Abstract

To measure patients' QOL in the daily practice of clinical oncology, we developed and tested the Care Notebook. This instrument has 24 questions expressed in single words or short phrases to make it more acceptable to patients. The Care Notebook, EORTC QLQ-C30 and FACIT-Sp-12 were administered to 249 outpatients with cancer. Construct validity was investigated by cluster analysis and multitrait scaling analysis. The results showed that three scales (physical well-being, mental well-being, and life well-being) could explain 55% of the variance in scores. The life well-being scale could be divided into subscales of Daily Functioning, Social Functioning, and Subjective QOL. Multitrait scaling analysis confirmed convergent and discriminant validity of these scales and subscales. Internal consistency and test-retest reliability were favorable. Differences in Care Notebook scores were also consistent with differences in performance status rating (known-groups validity), and Care Notebook scores correlated with EORTC QLQ-C30 and FACT-Sp-12 scores (concurrent validity). The Care Notebook allows clinical oncologists to easily collect valid and reliable QOL information of physical, mental, and life well-being repeatedly and with minimal burden on patients.

Key words: Care Notebook, Life well-being, Quality of life, Questionnaire, Subjective well-being

Abbreviations: CORE – Center on Outcomes, Research, and Education; ECOG – Eastern Cooperative Oncology Group; EORTC – European Organization for Research and Treatment of Cancer; EORTC QLQ-C30 – EORTC core questionnaire; FACT-G – Functional Assessment of Cancer Therapy Scale-General; FACIT-Sp-12 – Functional Assessment of Chronic Illness Therapy – The 12-item Spiritual Well-Being Scale; PSR – Performance Status Rating; QOL – Quality of Life; QOL-ACD – Quality of Life Questionnaire for Cancer Patients Treated with Anticancer Drugs

Introduction

Instruments to assess Quality of Life (QOL) in cancer patients have been developed worldwide. Such instruments include the European Organization for Research and Treatment of Cancer core questionnaire (EORTC QLQ-C30) [1], the Functional Assessment of Cancer Therapy Scale-General (FACT-G) [2], and the Quality of Life Questionnaire for Cancer Patients Treated with Anticancer Drugs (QOL-ACD) [3]. In clinical trials of anti-cancer therapy, QOL is increasingly seen as an important endpoint, and is often measured in addition to survival, tumor response, and toxicity [4].

We developed a Japanese questionnaire (the QOL-ACD); also, in cooperation with the original

developers of the EORTC QLQ-C30 and the FACT-G, we developed and tested Japanese versions of those instruments. The results showed that the EORTC QLQ-C30, FACT-G, and QOL-ACD could provide valid and reliable information on Japanese patients with cancer [5–7]. However, we found that patients often have difficulty completing these questionnaires during routine clinical and palliative care. Some cancer patients were uncomfortable routinely responding to certain items (for example, items that evoked their fear of death). Completing these questionnaires also seemed to be particularly difficult for patients with poor performance status rating (PSR). In our experience [5], a total of 444 EORTC QLQ-C30 questionnaires were administered on 105 inpatients (average 4.2/patient); 370 were completed, an overall return rate of 83%. Although the return rate for PS 0–2 patients was over 99% (225/228), the corresponding rates for PS 3 and PS 4 were 81% (38/47) and 13% (9/69), respectively [5]. Furthermore, because scale scores must be computed and because the scoring must account for the polarity of the wording of each item and response choice (positive or negative), doctors and nurses found the data from these instruments difficult to use in their daily clinical practice.

Measuring QOL can have clinical benefits. QOL questionnaire uses include fostering patient-provider communication. It can help clinicians and patients to identify problems and set priorities, and to assess therapy, palliative care, and rehabilitation [8]. However, measuring QOL in clinically useful ways is not simple. To the instrument developer, the combination of clinical relevance and ease of use for both patients and clinicians is elusive. Greenfield identified two barriers to clinical use of QOL measurement: problems with the meaning and interpretation of health-status scores, and problems with utilization and mainstreaming. The latter involves all of the issues associated with changing the day-to-day behavior of clinicians and providers' routine processes to facilitate routine use of health status measures in clinical settings [9]. Similar barriers were identified by Deyo: the need to process data quickly and the need for the results to be relevant to clinical practice [10].

We therefore developed the Care Notebook, a QOL instrument that we intended to be brief, valid, reliable, easy to administer and score, and clinically useful [11].

Methods and patients

Development of the care notebook

For version 1 of the Care Notebook [12], items that seemed to influence daily life were collected from doctors, nurses, and cancer patients. The number of items was reduced according to the method of conceptual analysis [13], and also according to the frequency with which such questions were asked by doctors and nurses in clinical practice. It was found that the clinicians routinely questioned patients first about physical conditions such as pain, shortness of breath, gastrointestinal symptoms, etc., and then about mental status such as mood and depression. After that, they sometimes asked patients about relationships with family and friends, and finally about global QOL. Version 1 of the Care Notebook consisted of those items, in the order used clinically (physical well-being, mental well-being, and life well-being). Each item in version 1 was written as a complete-sentence question, with a 5-point response scale for the items of physical well-being and mental well-being, and a 10-point scale for those of life well-being.

During their encounters with patients, clinicians' questions were usually not complete sentences but only a single word or a short phrase. Therefore, version 2 of the Care Notebook included an introductory instruction followed by questions written as single words or short phrases. Items concerning spirituality were lacking in version 1, but we observed that clinicians sometimes asked these questions at the end of an interview. Items concerning spirituality were added in version 2. An 11-point scale for all the items was used for the responses. Version 2 was tested with 40 cancer patients to seek face validity, and the results were used for further refinements that led to version 3.

Structure of the care notebook (version 3)

The instrument begins with very brief instructions, which are followed by question-items in three sections: 10 items on symptoms and physical conditions (particularly those that can be affected by medical treatment), six items on moods and psychological status, and eight items related to functioning and life situations (two items each on daily

physical functioning, social functioning, QOL and satisfaction, and spirituality). Each item is presented as a question consisting of one word or a short phrase. The patients respond using an 11-point scale. Finally, there is one section for unstructured responses to the question 'How might we help you improve your health and life?' (Appendix).

Sample and protocol

In this study, version 3 of the Care Notebook was tested in an outpatient clinic. The subjects were cancer patients who used the outpatient clinic of Saitama Cancer Center in March 2001. After informed consent was obtained, two sets of the Care Notebook, EORTC QLQ-C30 (1), and Functional Assessment of Chronic Illness Therapy – Spiritual well-being – The 12-item Spiritual well-being scale (FACIT-Sp-12) [14] were delivered, and answered twice at home, at an interval of 4 weeks. The patients submitted their completed questionnaires to our QOL Center without their doctor's participation. All of the procedures followed were in accordance with the Helsinki Declaration (1964, amended in 1975 and 1983) of the World Medical Association, and the protocol was approved by the Institutional Review Board of Saitama Cancer Center.

Analyses

For item convergent and discriminant validity, cluster analysis and a multi-trait scaling analysis [15, 16] were conducted. Scales and subscales were extracted by cluster analysis using the SAS program and were displayed in a dendrogram. The multi-trait scaling analysis was carried out to evaluate the extracted scale structure of the questionnaire. This technique to test discriminative validity is based on the examination of item-scale correlations. Namely, Pearson's correlations of an item with its own scale (corrected for overlap) and other scales were calculated. Item discriminant validity was supported by a comparison of the degree of correlation of an item with its own scale as compared with other scales. A scaling error was suspected when correlation of an item with another scale exceeded the correlation with its own scale [1].

The internal consistency of each scale was estimated with Cronbach's alpha [17]; a value of 0.70 or greater was considered to indicate acceptable internal consistency. Pearson's correlation coefficient was computed for test-retest reliability, and also for correlations with scores on the EORTC QLQ-C30 and FACIT-Sp-12 (concurrent validity). Student's *t*-test was used to compare the Care Notebook scores of groups with differing PSR scores (known-groups validity).

Results

Of the 266 cancer patients who used our outpatient clinic from the third to the fourth week in March 2001, 17 refused to participate in this study. Characteristics of the 249 participating patients are shown in Table 1. There were slightly more men

Table 1. Demographic and clinical characteristics (n = 249)

Variable	Category	Number	Percent	Missing
Sex	Male/female	139 / 101	(58 / 42)	9
Age	≤ 49 / 50–59 / 60–69 / ≥70	47 / 76 / 76 / 41	(20 / 32 / 32 / 17)	9
ECOG PS	0 / 1 / 2 / 3 or 4	177 / 55 / 11 / 5	(71 / 22 / 4 / 2)	9
Diagnosis	Breast cancer	70	(31)	25
	Lung cancer	50	(22)	
	Gastrointestinal cancer	40	(18)	
	Pharyngeal cancer	16	(7)	
	Lymphoma	14	(6)	
	Hepatoma	13	(6)	
	Others	17	(8)	
Stage	I / II / III / IV	20 / 49 / 56 / 56	(11 / 27 / 31 / 31)	68
Cancer disclosure	Yes/no	238 / 8	(97 /3)	3

than women. Most had progressive disease (stage III, 56 patients; stage IV, 56 patients), but had good PSR (PSR 0 or 1, 232 patients; PS 2–4, 16 patients). Almost all of the patients knew their diagnosis.

The dendrogram shows that three scales (which we call physical well-being, mental well-being, and life well-being) can explain slightly more than 50% of the total variance (Figure 1). At 75% of the total variance, life well-being was divided into three subscales (Daily Functioning, Social Functioning, and Subjective QOL). The Subjective-QOL subscale comprised four items (concerning global QOL, satisfaction, happiness, and spirituality).

With the scales described above, the multi-trait scaling analysis showed that all correlations of items with their own scale were above 0.40, indicating satisfactory item-convergent validity [1]. In the test of item-discriminant validity, one scaling error on the Appetite-Loss subscale was noted, and two scaling errors were found in the Social Functioning subscale. The rate of scaling error was 2.4% (3/126).

For test-retest reliability, Pearson's correlation coefficients for all the subscales and for the single-item symptoms were above 0.4. Cronbach's alpha coefficients for physical well-being, mental well-being, and life well-being were 0.86, 0.93, and 0.91, respectively (Table 2), indicating satisfactory internal consistency. Cronbach's alpha coefficients for all the subscales (appetite loss, constipation, fatigue, daily functioning, social functioning, and subjective QOL) showed satisfactory internal consistency (0.73 and higher).

Of the six (sub)scales tested, scores on five differed significantly between patients with a PSR of

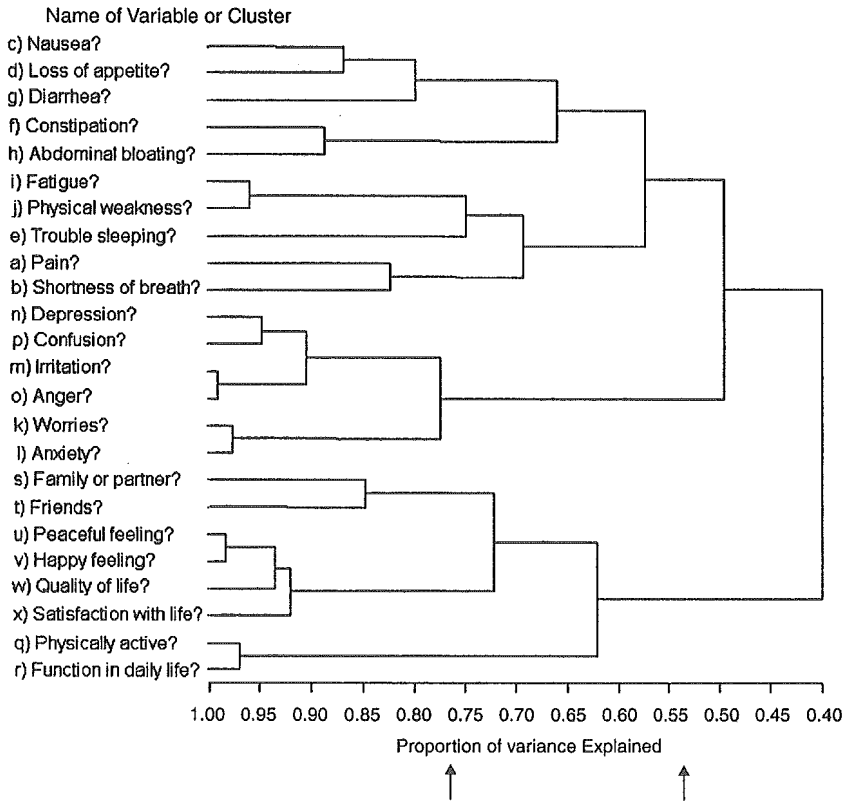

Figure 1. The dendrogram by cluster analysis shows that nine subscales can explain 75% of the total variance. The subscales can be named pain and shortness of breath, appetite loss, trouble sleeping, constipation, fatigue, mental well-being, daily functioning, social functioning, and subjective QOL. Slightly more than 50% of the total variance can be explained by three scales (interpreted as physical well-being, mental well-being, and life well-being). Therefore, life well-being scale is considered to have three subscales: daily functioning, social functioning, and subjective QOL.

Table 2. Reliability

Scale Subscale	Item	Cronbach's alpha	Test–retest reliability	Mean (SD)[a]
Physical well-being	(n = 10)	0.86		
Pain	A	–	0.66	1.63(2.39)
Shortness of breath	B	–	0.66	1.90(2.61)
Appetite loss	C, D, G	0.74	0.46	1.21(2.15)
Trouble sleeping	E	–	0.65	1.76(2.59)
Constipation	F, H	0.77	0.50	1.47(2.35)
Fatigue	I, J	0.88	0.65	2.59(2.73)
Mental well-being	K–P (n = 6)	0.93	0.68	2.31(2.54)
Life well-being	(n = 8)	0.91		
Daily functioning	Q, R	0.92	0.61	6.28(2.56)
Social functioning	S, T	0.73	0.40	8.07(2.36)
Subjective QOL	U–X	0.91	0.57	7.05(2.38)

[a]Lower scores represent lesser problems in physical and mental well-being. In life well–being, higher scores represent better QOL.

Table 3. Clinical validity: Known-groups comparison with PSR

Scale Subscale	PSR 0 Score (SE)	PSR 1- Score (SE)	*t*-test *P* value
Physical well-being	1.58 (0.13)	2.70 (0.29)	< 0.0001
Mental well-being	2.14 (0.18)	3.05 (0.31)	0.0089
Life well-being	7.36 (0.14)	6.53 (0.24)	0.0027
Daily functioning	6.69 (0.18)	5.27 (0.32)	< 0.0001
Social functioning	8.20 (0.16)	7.72 (0.31)	0.13
Subjective QOL	7.33 (0.16)	6.49 (0.26)	0.007

0 and those with a PSR greater than 0 (known-groups validity, Table 3). As expected, changes in PSR were not associated with Social Functioning. Scores on the Care Notebook scales and subscales correlated modestly or well with those on the EORTC QLQ-C30 and the FACIT-Sp-12; the only exceptions were low correlations of the Social Functioning subscale and of the item regarding diarrhea (concurrent validity, Table 4).

Discussion

Maintaining or improving patients' QOL is increasingly seen as important [8]. However, investigating patients' QOL in a busy clinical practice is difficult because the necessary steps require time, thought, recording, and follow-up [18]. The main barriers to collecting QOL data are logistic and the challenge remains to develop a method of collecting and analyzing QOL information in a manner which enhances decision making [19]. Detmar used the EORTC QLQ-C30 in clinical practice. The responses were scored by computer and displayed graphically, and both physicians and patients could see the results before the consultation. This helped them discuss HRQL issues and raised the physicians' awareness of their patients' HRQL [20]. Having similar goals with respect to clinical decision-making, and having experiences with the logistics of collecting QOL data, we developed the Care Notebook, which needs no specialized automated system using computer.

In developing version 1, we expected to measure five domains: global QOL, and physical, mental, functional, and social well-being. But the result of factor analysis with varimax rotation showed only three factors: physical well-being, mental well-being, and one factor combining functional well-being, social well-being, and global QOL [12]. In interpreting this result, we suspected that the patients assigned different weights to the five domains. Specifically, we hypothesized that these patients considered a larger domain, which we called "life well-being," to be as

Table 4. Concurrent validity with the EORTC QLQ-C30 and the FACT-Sp

Care Notebook Scale, Subscale	QLQ-C30 Scale/Item*	EORTC QLQ-C30**	FACIT -Sp-12**
Physical well-being			
Pain	Pain	**0.62**	
Shortness of breath	Dyspnea	**0.63**	
Appetite loss	Appetite loss	**0.49**	
	Nausea/vomiting	**0.46**	
	Diarrhea	0.36	
Trouble sleeping	Sleep disturbance	**0.47**	
Constipation	Constipation	**0.58**	
Fatigue	Fatigue	**0.57**	
Mental well-being	EF	**0.71**	**0.58**
Life well-being	QL	**0.55**	**0.55**
Daily functioning	PF	**0.51**	**0.46**
	RF	**0.54**	
Social functioning	SF	0.22	0.34
Subjective QOL	QL	**0.47**	**0.58**

* EF–Emotional functioning; QL–Global quality of life; PF–Physical functioning; RF–Role functioning; SF–Social functioning.
** The values of Pearson's correlation coefficients more than 0.40 are bold.

important as physical well-being and mental well-being, and that this larger domain comprised functional well-being, social well-being, and global QOL.

Therefore, we used cluster analysis in this study for version 3. This analysis resembles exploratory factor analyses in which the number of factors can be changed. As expected, slightly more than 50% of the variance could be explained by three scales: physical well-being, mental well-being, and life well-being. Increases in the percentage of the variance explained accompany increases in the number of factors (scales). In this case, at 75% of the variance explained, the Life Well-Being scale divided into subscales, which we call daily functioning, social well-being, and subjective QOL. In addition to this forward-looking cluster analysis, we used multitrait scaling analysis as a backward-looking proof. The multitrait analysis confirmed convergent and discriminant validity of these scales and subscales. The scales and subscales were also internally consistent.

Because items in the Subjective QOL subscale ask about "satisfaction with life", "quality of life", "peaceful feeling", and "happy feeling", that subscale may be similar to "subjective well-being" as described by Diener et al. [21]. Life well-being is somewhat broader. It also includes social functioning and daily functioning. The dendrogram suggests that outpatients with cancer can distinguish life well-being from their physical and psychological signs and symptoms (Figure 1). Further studies of this concept should contribute to a deeper understanding of QOL in patients with cancer.

In the validation study, test-retest reliability was not high. Correlation coefficients were above only 0.4. This was assessed over a 4-week interval, so differences in test and retest scores may have been related to important changes in disease status and HRQL. After this study, we clinically used the Care Notebook with inpatients at the Saitama Cancer Center who had lung cancer (n = 93; 79 men and 14 women). They answered the Care Notebook questions every Monday morning (median number of Mondays, 3; range, 1–16). Of the 93 patients, 68 had no change in PSR, so we used data from those 68 to calculate test-retest reliability over a 1-week interval. With only one exception, all the correlation coefficients were above 0.7 (pain: 0.80, shortness of breath: 0.94, appetite loss: 0.87, trouble sleeping: 0.77, constipation: 0.67, fatigue: 0.93, mental well-being: 0.92, daily functioning: 0.81, social functioning: 0.95, subjective QOL: 0.88).

Concurrent validity of the Care Notebook, the EORTC QLQ-C30, and the FACIT-Sp-12 indicate that these instruments can measure some of the same domains, even though they were developed by different methods. The only apparent problem in concurrent validity was with the social-functioning subscale: the correlation between the measured values was low (0.22) (Table 4). Both the Care Notebook and the EORTC QLQ-C30 measure 'social-functioning', but the items are quite different. The Care Notebook asks in general about relationships with 'friends' and with 'family or partner'; it does not specifically mention any possible effects of illness or of therapy. In contrast, the EORTC QLQ-C30 asks specifically about illness-related interference with "social activities" and with "family life". That difference in content may account for the low correlation. In this case, the scales have the same names despite important differences in their underlying concepts.

The Care Notebook fills a need in the daily practice of clinical oncology. By including items frequently asked by clinicians, and by writing them as single words or short phrases, we sought to obtain, in an easily repeated way, information about QOL that oncology clinicians seek in their regular interactions with patients. To elicit expressions of any concerns that are not directly addressed in the list of question-items, we included an open-ended question. We also designed the physical layout of the instrument so that it is easy to handle, and so that one volume can contain many response sheets, each of which can be separated from the others and stored in the patient's records [22].

Of the 93 inpatients mentioned above, data were obtained from 34 such patients whose PSR became 3 or 4 during their hospitalization, which indicates that the questionnaire could be used by patients with a poor performance status. Each Monday afternoon, the doctors and nurses discussed the information in each patient's Care Notebook. The unipolarity in location of the response choices facilitated these discussions (Appendix). Because problems are always indicated by circles toward the left side of the page, with just a quick glance at the response form the doctors and nurses could decide whether or not to investigate the patient's situation further. The Care Notebook also includes an open-ended question. We found that patients sometimes responded to this question, and their responses were generally of four types: conditions and mental problems to be resolved, help for improvement in daily life, questions regarding the disease (diagnosis, treatments, prognosis), and private messages from patients to clinicians. The last category includes patients' last wills, decisions regarding the future, and messages of thanks from patients to clinicians. Information discussed using Care Notebook was immediately employed in clinical practice. For examples, the doctors and nurses could make decisions with regard to treatments for pain and depression. Such decisions could be made and implemented relatively quickly.

As indicated by the previous studies using the EORTC QLQ-C30 [20, 23, 24], incorporating HRQL assessments in daily clinical oncology practice is clearly important, but the burdens these assessments impose on patients and clinicians should be minimized. The Care Notebook's brevity, validity, and reliability, together with its ease of administration, and interpretation might make it useful in clinical oncology. Its "low-tech" simplicity could also prove to be important in settings with limited financial resources. Further research testing its usefulness in routine clinical practice will be needed.

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Appendix A: Care Notebook (version 3)

As the response choices for the items are the unipolarity *in location*, answering page, i.e. the right page of the Care Notebook, presents patient' information like a graphic showing by itself. When scoring, the Care Notebook is designed to be calculated on an item-by-item basis, a subscale-score basis and a scale-score basis. Single items of pain, shortness of breath and trouble sleeping are items a, b and e, respectively. The three scales are physical well-being (items a–j), mental well-being (items k–p) and life well-being (items q–x), and the multi-item subscales are appetite loss (items c, d, and g), constipation (items f and h), fatigue (items i and j), daily functioning (items q and r), social functioning (items s and t), subjective QOL (items u–x). The score of a subscale or a scale is calculated by the sum of the item-scores per the number of the items. Lower scores represent lesser problems in physical and mental well-being. In life well-being, higher scores represent better QOL.

Although intellectual property is protected, readers can freely use and modify this instrument without permission for clinical purposes. For research, if readers describe using the Care Notebook or its constructs in their presentation or in the text of their article, then they can use and modify this instrument without permission. This instrument and further information can be obtained from <homepage3.nifty.com/care-notebook/>.

Appendix B

Thinking about the past week, please answer the questions below by circling one number in each row on the right-hand page.

Month Day Year Name

I. Did you have any of these problems?

Please circle the number that best applies to you, using 10 for the worst condition.

I. Symptoms

a)	Pain?	a)	severe pain	10	9	8	7	6	5	4	3	2	1	0	none at all
b)	Shortness of breath?	b)	severe shortness of breath	10	9	8	7	6	5	4	3	2	1	0	none at all
c)	Nausea?	c)	severe nausea	10	9	8	7	6	5	4	3	2	1	0	none at all
d)	Loss of appetite?	d)	complete loss of appetite	10	9	8	7	6	5	4	3	2	1	0	good appetite
e)	Trouble sleeping?	e)	severe trouble sleeping	10	9	8	7	6	5	4	3	2	1	0	none at all
f)	Constipation?	f)	severe constipation	10	9	8	7	6	5	4	3	2	1	0	none at all
g)	Diarrhea?	g)	severe diarrhea	10	9	8	7	6	5	4	3	2	1	0	none at all
h)	Abdominal bloating?	h)	severe abdominal bloating	10	9	8	7	6	5	4	3	2	1	0	none at all
i)	Fatigue?	i)	severe fatigue	10	9	8	7	6	5	4	3	2	1	0	none at all
j)	Physical weakness?	j)	severe weakness	10	9	8	7	6	5	4	3	2	1	0	none at all

II. Did you have any of these problems with feelings or moods?

Please circle the number that best applies to you, using 10 for the worst condition.

II. Problems with feelings or moods

k)	Worries?	k)	many worries	10	9	8	7	6	5	4	3	2	1	0	none at all
l)	Anxiety?	l)	severe anxiety	10	9	8	7	6	5	4	3	2	1	0	none at all
m)	Irritation?	m)	severe irritation	10	9	8	7	6	5	4	3	2	1	0	none at all
n)	Depression?	n)	severe depression	10	9	8	7	6	5	4	3	2	1	0	none at all
o)	Anger?	o)	intense anger	10	9	8	7	6	5	4	3	2	1	0	none at all
p)	Confusion?	p)	severe confusion	10	9	8	7	6	5	4	3	2	1	0	none at all

III. How was your life over the past week?

Please circle the number that best applies to you, using 10 for the BEST condition.

III. Your life over the past week

q)	Ability to be physically active?	q)	unable	0	1	2	3	4	5	6	7	8	9	10	very able to be physically active
r)	Ability to function in daily life?	r)	unable	0	1	2	3	4	5	6	7	8	9	10	very able to function in daily life
s)	Relationship with your family or partner?	s)	the worst	0	1	2	3	4	5	6	7	8	9	10	the best relationship with my family/partner
t)	Relationship with your friends?	t)	the worst	0	1	2	3	4	5	6	7	8	9	10	the best relationship with my friends
u)	Peaceful feeling?	u)	none at all	0	1	2	3	4	5	6	7	8	9	10	the most peaceful feeling
v)	Happy feeling?	v)	none at all	0	1	2	3	4	5	6	7	8	9	10	the happiest feeling
w)	Quality of life?	w)	the worst	0	1	2	3	4	5	6	7	8	9	10	the best quality of life
x)	Satisfaction with life (considering your condition and treatment)?	x)	none at all	0	1	2	3	4	5	6	7	8	9	10	the most satisfaction with life

IV. Please tell us how we might help you improve your health and life.

IV. How might we help you improve your health and life?

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Significance of Valine/Leucine^{247} Polymorphism of β_2-Glycoprotein I in Antiphospholipid Syndrome

Increased Reactivity of Anti–β_2-Glycoprotein I Autoantibodies to the Valine^{247} β_2-Glycoprotein I Variant

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Objective. **To clarify the consequences of the valine/leucine polymorphism at position 247 of the β_2-glycoprotein I (β_2GPI) gene in patients with antiphospholipid syndrome (APS), by investigating the correlation between genotypes and the presence of anti-β_2GPI antibody. The reactivity of anti-β_2GPI antibodies was characterized using recombinant Val^{247} and Leu^{247} β_2GPI.**

Methods. **Sixty-five Japanese patients with APS and/or systemic lupus erythematosus who were positive for antiphospholipid antibodies and 61 controls were analyzed for the presence of the Val/Leu^{247} polymorphism of β_2GPI. Polymorphism assignment was determined by polymerase chain reaction followed by restriction enzyme digestion. Recombinant Val^{247} and Leu^{247} β_2GPI were established to compare the reactivity of anti-β_2GPI antibodies to β_2GPI between these variants. The variants were prepared on polyoxygenated plates or cardiolipin-coated plates, and the reactivity of a series of anti-β_2GPI antibodies (immunized anti-human β_2GPI monoclonal antibodies [Cof-19–21] and autoimmune anti-β_2GPI monoclonal antibodies [EY1C8, EY2C9, and TM1G2]) and IgGs purified from patient sera was investigated.**

Results. **A positive correlation between the Val^{247} allele and the presence of anti-β_2GPI antibodies was observed in the patient group. Human monoclonal/polyclonal anti-β_2GPI autoantibodies showed higher binding to recombinant Val^{247} β_2GPI than to Leu^{247} β_2GPI, although no difference in the reactivity of the immunized anti-β_2GPI between these variants was observed. Conformational optimization showed that the replacement of Leu^{247} by Val^{247} led to a significant alteration in the tertiary structure of domain V and/or the domain IV–V interaction.**

Conclusion. **The Val^{247} β_2GPI allele was associated with both a high frequency of anti-β_2GPI antibodies and stronger reactivity with anti-β_2GPI antibodies compared with the Leu^{247} β_2GPI allele, suggesting that the Val^{247} β_2GPI allele may be one of the genetic risk factors for development of APS.**

The antiphospholipid syndrome (APS) is characterized by arterial/venous thrombosis and pregnancy morbidity in the presence of antiphospholipid antibodies (aPL) (1–3). Among the targets of aPL, β_2-glycoprotein I (β_2GPI), which bears epitopes for anticardiolipin antibodies (aCL), has been extensively studied (4–6). APS-related aCL do not recognize free β_2GPI, but do recognize β_2GPI when it is complexed with phospholipids or negatively charged surfaces, by exposure of cryptic epitopes (7) or increment of antigen density (8).

The significance of antigen polymorphism in the production of autoantibodies or the development of autoimmune diseases is now being widely discussed. It is speculated that amino acid substitution in antigens can lead to differences in antigenic epitopes of a given protein. In particular, β_2GPI undergoes conformational

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alteration upon interaction with phospholipids (9). β_2GPI polymorphism on or near the phospholipid binding site can affect the binding or production of aCL (anti-β_2GPI autoantibodies), the result being altered development of APS. Polymorphism near the antigenic site, or which leads to alteration of the tertiary structure of the whole molecule, may affect the binding of autoantibodies. Five different gene polymorphisms of β_2GPI attributable to a single-nucleotide mutation have been described: 4 are a single amino acid substitution at positions 88, 247, 306, and 316 (10), and the other is a frameshift mutation associated with β_2GPI deficiency found in the Japanese population (11). In particular, the Val/Leu^{247} polymorphism locates in domain V of β_2GPI, between the phospholipid binding site in domain V and the potential epitopes of anti-β_2GPI antibodies in domain IV, as we reported previously (12). Although anti-β_2GPI antibodies are reported to direct to domain I (13) or domain V (14) as well, it should be considered that a certain polymorphism alters the conformation of the molecule, affecting function or antibody binding at a distant site.

We previously reported that, in a group of British Caucasian subjects, the Val^{247} allele was significantly more frequent in primary APS patients with anti-β_2GPI antibodies than in controls or in primary APS patients without anti-β_2GPI antibodies (15), but the importance of the Val^{247} allele in patients with APS is still controversial. In this study, we analyzed the correlation between the β_2GPI Val^{247} allele and anti-β_2GPI antibodies in the Japanese population. We also investigated the reactivity of anti-β_2GPI antibodies to recombinant Val^{247} β_2GPI and Leu^{247} β_2GPI, using a series of monoclonal anti-β_2GPI antibodies and IgGs purified from sera of patients with APS. Finally, to investigate the difference in anti-β_2GPI binding to those variants, we conformationally optimized to domain V and the domain IV–V complex of β_2GPI variants at position 247, referring the crystal structure of β_2GPI.

PATIENTS AND METHODS

Patients and controls. The study group comprised 65 patients (median age 38 years [range 18–74 years]; 57 women and 8 men) who attended the Hokkaido University Hospital, all of whom were positive for aPL (IgG, IgA, or IgM class aCL, and/or lupus anticoagulant). Thirty-four patients had APS (16 had primary APS, and 18 had secondary APS), and 31 patients did not have APS (24 had systemic lupus erythematosus [SLE], and 7 had other rheumatic diseases). Among all subjects, 19 had a history of arterial thrombosis, and 6 had venous thrombosis. Of the 31 patients with a history of pregnancy, 8 experienced pregnancy complications (some patients had more than 1 manifestation of pregnancy morbidity). Anti-β_2GPI antibodies were detected by enzyme-linked immunosorbent assay (ELISA) as β_2GPI-dependent aCL (16). IgG, IgA, or IgM class β_2GPI-dependent aCL were found in 30, 14, and 21 patients, respectively (some patients had >1 isotype), and 34 patients had at least 1 of those isotypes. Lupus anticoagulant, detected by 3 standard methods described previously (17), was found in 51 patients. The diagnoses of APS and SLE, respectively, were based on the preliminary classification criteria for definite APS (18) and the American College of Rheumatology criteria for the classification of SLE (19). Informed consent was obtained from each patient or control subject. The control group comprised 61 healthy individuals with no history of autoimmune, thrombotic, or notable infectious disease.

Determination of β_2GPI gene polymorphism. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using a standard phenol–chloroform extraction procedure or the DnaQuick kit (Dainippon, Osaka, Japan). Polymorphism assignment was determined by polymerase chain reaction (PCR) followed by allele-specific restriction enzyme digestion (PCR–restriction fragment length polymorphism) using *Rsa* I (Promega, Southampton, UK) as described previously (15).

Purification of patient IgG. Sera from 11 patients positive for IgG class β_2GPI-dependent aCL were collected. The mean (±SD) titer of aCL IgG from these patients was 29.0 ± 21.5 IgG phospholipid (GPL) units (range 12.4 to >98 GPL units). IgG was purified from these sera using a protein G column and the MAbTrap GII IgG purification kit (Pharmacia Biotech, Freiburg, Germany), as recommended by the manufacturer.

Monoclonal anti-β_2GPI antibodies. Two types of anti-β_2GPI monoclonal antibodies were used. Cof-19, Cof-20, and Cof-21 are mouse monoclonal anti-human β_2GPI antibodies obtained from immunized BALB/c mice, directed to domains V, III, and IV of β_2GPI, respectively. These monoclonal antibodies recognize the native structure of human β_2GPI (12).

EY1C8, EY2C9, and TM1G2 are IgM class autoimmune monoclonal antibodies established from patients with APS (20). These antibodies bind to domain IV of β_2GPI, but only after interaction with solid-phase phospholipids or with a polyoxygenated polystyrene surface. EY1C8 and EY2C9 were established from a patient whose genotype of β_2GPI was heterozygous for Val/Leu^{247}. The genotype of the patient with TM1G2 was not determined.

Preparation of recombinant β_2GPI. As previously reported, genes were expressed in *Spodoptera frugiperda* Sf9 insect cells infected with recombinant baculoviruses (12). A full-length complementary DNA of human β_2GPI coding Val^{247} was originally obtained from Hep-G2 cells (21), and the valine residue was replaced by leucine, using the GeneEditor in vitro Site-Directed Mutagenesis System (Promega, Madison, WI). The sequence of the primers for a mutant Val^{247}→Leu (GTA→TTA) is as follows: 5′-GCATCTTGTAAATTACCTGTGAAAAAAG-3′. A DNA sequence of the mutant was verified by analysis using ABI Prism model 310 (PE Applied Biosystems, Foster City, CA).

Binding assays of monoclonal anti-β_2GPI antibodies and purified IgGs to the recombinant β_2GPI (cardiolipin-coated plate). The reactivity of a series of monoclonal anti-β_2GPI antibodies and IgG fractions (purified from the sera of APS patients positive for IgG class anti-β_2GPI) against 2 β_2GPI variants was investigated using an ELISA. ELISAs were performed using a cardiolipin-coated plate as previously reported (16) but with a slight modification. Briefly, the wells of Sumilon Type S microtiter plates (Sumitomo Bakelite, Tokyo, Japan) were filled with 30 μl of 50 μg/ml cardiolipin (Sigma, St. Louis, MO) and dried overnight at 4°C. After blocking with 2% gelatin in phosphate buffered saline (PBS) for 2 hours and washing 3 times with 0.05% PBS–Tween, 50 μl of 10 μg/ml recombinant β_2GPI and controls were distributed and incubated for 30 minutes at room temperature. Wells were filled with 50 μl of serial dilutions of monoclonal antibodies (Cof-19–21, EY1C8 and EY2C9, and TM1G2) or purified patient IgG (100 μg/ml), followed by incubation for 30 minutes at room temperature. After washing 3 times, 50 μl of alkaline phosphatase–conjugated anti-mouse IgG (1:3,000), anti-human IgM (1:1,000), or anti-human IgG (1:6,000) was distributed and incubated for 1 hour at room temperature. The plates were washed 4 times, and 100 μl of 1 mg/ml *p*-nitrophenyl phosphate disodium (Sigma) in 1*M* diethanolamine buffer (pH 9.8) was distributed. Optical density (OD) was read at 405 nm, with reference at 620 nm. One percent fatty acid–free bovine serum albumin (BSA) (A-6003; Sigma)–PBS was used as sample diluent and control.

Binding assays of monoclonal anti-β_2GPI antibodies to recombinant β_2GPI (polyoxygenated plate). Anti-β_2GPI antibody detection assay using polyoxygenated plates was performed as previously reported (22), with minor modifications. Briefly, the wells of polyoxygenated MaxiSorp microtiter plates (Nalge Nunc International, Roskilde, Denmark) were coated with 50 μl of 1 μg/ml recombinant β_2GPI in PBS and incubated overnight at 4°C. After blocking with 3% gelatin–PBS at 37°C for 1 hour and washing 3 times with PBS–Tween, 50 μl of monoclonal antibodies, diluted with 1% BSA–PBS, were distributed and incubated for 1 hour at room temperature. The following steps were taken, in a similar manner.

Conformational optimization of domain V and the domain IV–V complex in human β_2GPI variants at position 247. A conformation of domain V in the valine variant at position 247 was first constructed from the crystal structure of the leucine variant (implemented in Protein Data Bank: 1C1Z) (23). Replacement of leucine by valine at position 247 was performed using the Quanta system (Molecular Simulations, San Diego, CA), and the model was optimized by 500 cycles of energy minimization by the CHARMm program (24), with hydrophilic hydrogen atoms and TIP3 water molecules (25). Molecular dynamics simulation (5 psec) of the model was then performed with 0.002 psec time steps. The cutoff distance for nonbonded interactions was set to 15Å, and the dielectric constant was 1.0. A nonbonded pair list was updated every 10 steps. The most stable structure of each domain in the dynamics iterations was then optimized by 500 cycles of energy minimization. The final structures of domain V consisted of 2,616 atoms, including 603 TIP3 water molecules, and had a total energy of -1.63×10^4 kcal/mole with a root-mean-square force of 0.869 kcal/mole.

Molecular models of a domain IV–V complex (leucine and valine variants at position 247) were further constructed by considering the location of the oligosaccharide attachment site in domain IV, the location of epitopic regions of the Cof-8 and Cof-20 monoclonal antibodies, the junction between domains IV and V, and molecular surface charges of both domains. These models were again optimized by molecular dynamics simulation and by energy minimization as described above. The final structures of the complex in the leucine and valine variants consisted of 3,773 and 3,778 atoms, respectively, including hydrophilic hydrogen atoms and 806 and 808 TIP3 water molecules, respectively, and had total energy of -2.07×104 and -2.03×104 kcal/mole with a root-mean-square force of 0.985 and 0.979 kcal/mole, respectively.

Statistical analysis. Correlations between the allele frequencies and clinical features such as the positiveness of β_2GPI-dependent aCL were expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs). *P* values were determined by chi-square test with Yates' correction. *P* values less than or equal to 0.05 were considered significant.

RESULTS

Val/Leu^{247} polymorphism of β_2GPI and the presence of β_2GPI-dependent aCL. As shown in Table 1, the Leu^{247} allele was dominant in the population of healthy Japanese individuals, compared with Caucasians, which is consistent with a previous report (26). Japanese patients with anti-β_2GPI had a significantly increased frequency of the Val^{247} allele, compared with Japanese patients without anti-β_2GPI ($P = 0.0107$) or Japanese controls ($P = 0.0209$).

The binding of autoimmune anti-β_2GPI to recombinant Val^{247} and Leu^{247} β_2GPI. Representative binding curves using cardiolipin-coated plates and polyoxygenated plates are shown in Figure 1. Regardless of the type of plates, Cof-20 bound equally to valine and leucine variants of β_2GPI (Figures 1a and c), in any concentration of Cof-20. The binding curves of Cof-19 and Cof-21 were similar to that of Cof-20 (results not

Table 1. Frequency of the Val^{247} allele of β_2GPI in patients with APS*

Group	Japanese	British Caucasians
Patients with anti-β_2GPI	23/68 (33.8)†	48/56 (85.7)‡
Patients without anti-β_2GPI	9/62 (14.5)	39/58 (67.2)
Controls	23/122 (18.9)	55/78 (70.5)

* Values are the number (%). β_2GPI = β_2-glycoprotein I; APS = antiphospholipid syndrome.
† $P = 0.0107$ versus patients without anti-β_2GPI (odds ratio [OR] 3.01, 95% confidence interval [95% CI] 1.26–7.16), and $P = 0.0209$ versus controls, by chi-square test (OR 2.15, 95% CI 1.09–4.23).
‡ $P = 0.204$ versus patients without anti-β_2GPI (OR 2.92, 95% CI 1.16–7.39), and $P = 0.0396$ versus controls, by chi-square test (OR 2.51, 95% CI 1.03–6.13).

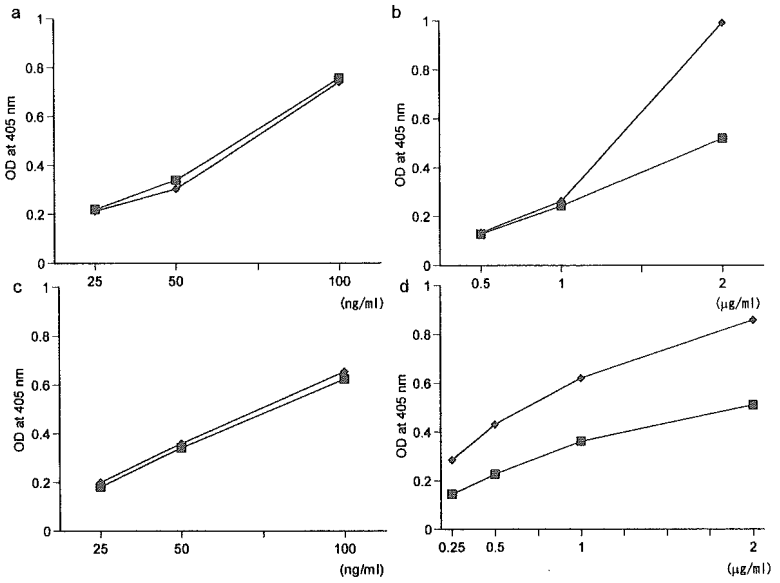

Figure 1. Representative binding curves of monoclonal anti-β_2-glycoprotein I (anti-β_2GPI) antibodies to recombinant valine/leucine^{247} β_2GPI. **a,** Binding curve of Cof-20 using cardiolipin-coated plate. **b,** Binding curve of EY2C9 using cardiolipin-coated plate. **c,** Binding curve of Cof-20 using polyoxygenated plate. **d,** Binding curve of EY2C9 using polyoxygenated plate. Binding to Val^{247} β_2GPI and Leu^{247} β_2GPI are indicated with diamonds and squares, respectively. OD = optical density.

shown). In contrast, EY2C9 showed stronger binding to Val^{247} β_2GPI than to Leu^{247} β_2GPI (Figures 1b and d). EY1C8 and TM1G2 also showed stronger binding to Val^{247} β_2GPI. Figure 2a shows the binding of the monoclonal antibodies, on cardiolipin-coated plates, in the following concentrations: for Cof-19–21, 100 ng/ml;

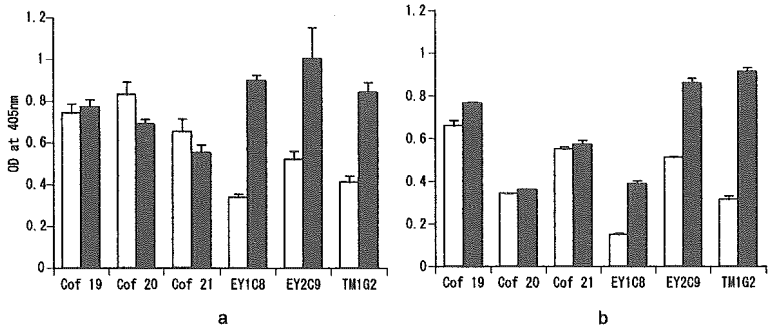

Figure 2. Reactivity of anti-β_2-glycoprotein I (anti-β_2GPI) antibodies to β_2GPI variants. **a,** The binding of monoclonal anti-β_2GPI antibodies to the recombinant valine/leucine^{247} β_2GPI was investigated using enzyme-linked immunosorbent assay (ELISA) on cardiolipin-coated plates. Concentrations of antigens and antibodies were as follows: for recombinant β_2GPI, 10 μg/ml; for Cof-19–21, 100 ng/ml; for EY1C8 and EY2C9, 2 μg/ml; for TM1G2, 5 μg/ml. **b,** The binding of monoclonal anti-β_2GPI antibodies to the recombinant Val/Leu^{247} β_2GPI was investigated using ELISA on polyoxygenated plates. Concentrations of antigens and antibodies were as follows: for recombinant β_2GPI, 1 μg/ml; for Cof-19–21, 50 ng/ml; for EY1C8 and EY2C9, 2 μg/ml; for TM1G2, 5 μg/ml. Results were presented as the optical density (OD) at 405 nm. Open columns indicate binding activity to Leu^{247} β_2GPI, and solid columns indicate binding activity to Val^{247} β_2GPI. Bars show the mean and SD.

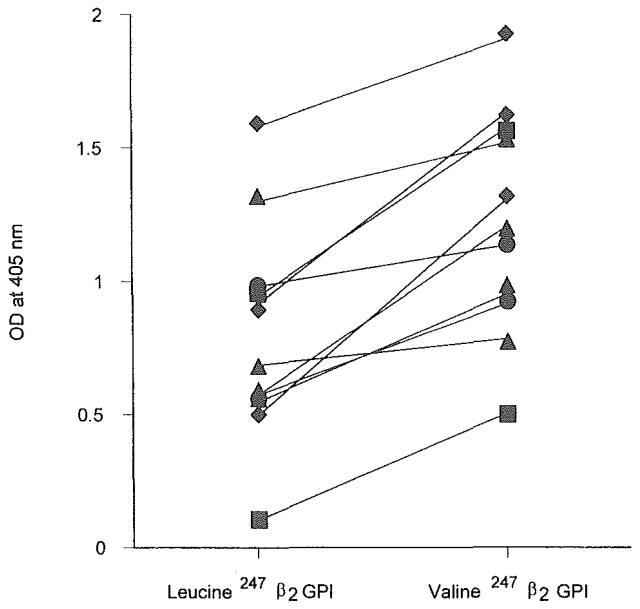

Figure 3. Reactivity of purified IgG from patients (100 μg/ml) to recombinant Val/Leu^{247} β_2-glycoprotein I (β_2GPI) (10 μg/ml), presented as the optical density (OD) at 405 nm. Squares, circles, and triangles indicate patients homozygous for the Leu^{247} allele, homozygous for the Val^{247} allele, and heterozygous for the Val/Leu^{247} allele, respectively. Diamonds indicate patients whose genotypes were not available.

for EY1C8 and EY2C9, 1 μg/ml; and for TM1G2, 2.5μg/ml. In contrast with the close reactivity of Cof-19, Cof-20, and Cof-21 between Val^{247} β_2GPI and Leu^{247} β_2GPI, autoimmune monoclonal antibodies (EY1C8, EY2C9, and TM1G2) showed higher binding to Val^{247} β_2GPI than to Leu^{247} β_2GPI. The autoimmune monoclonal antibodies also showed a higher binding to Val^{247} β_2GPI directly coated on polyoxygenated plates (Figure 2b). IgG in sera collected from 11 patients (100 μg/ml) also showed higher binding to Val^{247} β_2GPI than to Leu^{247} β_2GPI on cardiolipin-coated plates, regardless of the patients' genotypes (Figure 3).

Conformational alteration by leucine replacement by valine at position 247. Each domain V conformation in 2 variants at position 247 is shown in Figure 4a. The root-mean-square deviations for matching backbone atoms and equivalent atoms in the leucine and valine variants were 0.76 and 1.11 Å, respectively. The largest shift was observed at Val^{303}, one of the residues located on the backbone neighboring position 247. The shift seemed to be caused by weak flexibility of side chains consisting of Val^{247}, Pro^{248}, and Val^{249} and the electrostatic interactions between Lys^{250}, Lys^{251}, Glu^{307}, and Lys^{308}.

The molecular models of the IV–V complex in leucine and valine variants are shown in Figure 4b. The root-mean-square deviations for matching these backbone atoms and equivalent atoms were 1.72 and 2.03 Å, respectively. Electrostatic interactions and hydrogen bonds between Asp^{193} and Lys^{246}/Lys^{250}, Asp^{222} and Lys^{305}, and Glu^{228} and Lys^{308} appeared in the IV–V complex, but the interaction between Glu^{228} and Lys^{308} was disrupted by the leucine replacement by valine, because direction of the Lys^{308} side chain was significantly changed in the complex. As a result, Trp^{235} of domain IV, located on the contact surface with domain V, was slightly shifted.

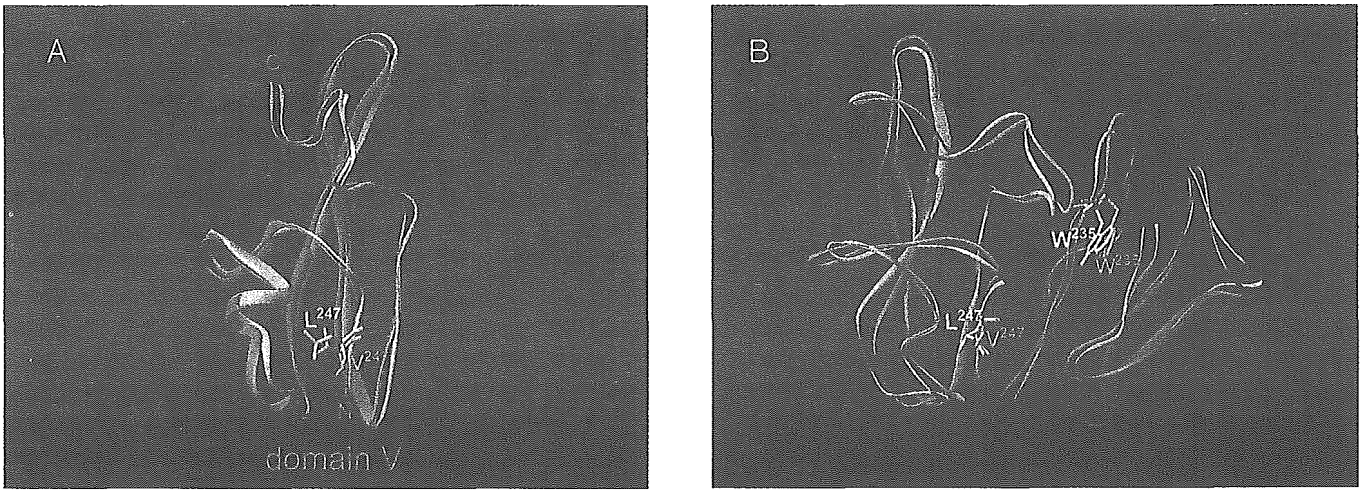

Figure 4. Conformational alterations in domain V (**A**) and in the domain IV–V complex (**B**), replacing leucine by valine at position 247. Structure of the valine (light blue) and leucine (white) variants was shown by a ribbon representation with the secondary structure.