hampered by fundamental conceptual and methodological issues.[2,16] Few studies, for example, have considered physical and mental health conditions as potential confounders. As shown in a recent German study, the relationship between sleep duration and body mass index (BMI) did not persist after controlling for self-rated physical health and emotional status.[21]

Moreover, the inconsistency might have been caused in part by the different definitions and measurements of obesity. BMI, an indicator of overall obesity, has been frequently used in previous studies to define weight gain and obesity. The importance of central obesity and abdominal fat mass has been known to have a stronger relation to the prevalence of each component of metabolic syndrome (hyperglycemia, diabetes and hypertension) than BMI.[22] Our previous study has indicated a superior performance of visceral fat area (VFA) to predict the clustering of metabolic risk factors compared with BMI, waist circumference (WC) and subcutaneous fat area (SFA) in a Japanese population.[23] It remains unclear, however, whether sleep duration is related to abdominal fat areas after taking potential confounders into account. The objective of this study was therefore to examine the relationship between sleep duration and BMI, WC, VFA and SFA in a large sample of Japanese working population enrolled in the Hitachi Health Study.

MATERIALS AND METHODS

Study procedure and subjects

This cross-sectional study was conducted in 2009 and 2010 during a comprehensive annual health examination conducted at the Hitachi Health Care Center, Ibaraki prefecture, Japan. The procedure of the study has been described elsewhere.[24,25] In brief, participants were asked to fill in a computer-based survey questionnaire on the day of the regular health checkup. In total, 17 606 male employees and their spouses underwent the checkup after having fasted overnight. Of these participants, 6537 subjects received an abdominal computed tomography (CT) scanning examination and were the targets for this study. We excluded subjects with a history of diabetes mellitus ($n=229$), stroke ($n=28$), myocardial infarction ($n=39$), cancer ($n=38$), psychiatric illnesses ($n=98$) and insomnia ($n=59$). We further excluded four subjects who did not provide information regarding sleep duration. Some subjects were overlapped and fell into more than one exclusion criteria. We finally included 6271 subjects in the analyses. We obtained written informed consent from each participant after the nature and possible consequences of the study had been fully explained. The study protocol was reviewed and approved by the Ethics Committee of the National Center for Global Health and Medicine, Tokyo.

Variables and measurements

Anthropometric and blood measurements. WC, VFA and SFA were measured by using a CT scanner, the details of which have been described elsewhere.[25] In brief, single slice imaging was performed at the umbilical level in a supine position (Redix Turbo; Hitachi Medico, Chiyoda-ku, Tokyo). The imaging conditions were 120 kV, 50 mA, with a slice thickness of 5 mm. WC, SFA and VFA were calculated by using the PC software application fatPointer (Hitachi Medico). Body height and weight were measured by using an automated scale (BF-220; TANITA; Itabashi-ku, Tokyo) with the subjects wearing a light gown. BMI was calculated as body weight in kg divided by the square of body height in meter.

Sleep duration. Sleep duration was self-reported. The average sleep duration on weekdays was defined by the response to the question (as translated into English): 'On average, how many hours do you sleep per day?' The response categories included: '<5 h', '5 to <6 h', '6 to <7 h' and '$\geqslant 7$ h'.

Confounding variables. Health-related lifestyles were ascertained by using a questionnaire. Participants entered their responses to the questionnaire directly into a computer using a custom-designed data entry system. Regarding the health conditions of participants , data were obtained from the routine health examination. Physical illness was defined as having been diagnosed with and/or being currently under treatment of at least one of the following diseases: hypertension, diabetes mellitus, hyperlipidemia, hyperuricemia, anemia, gastric ulcer, duodenal ulcer, colon polyp, chronic hepatitis, fatty liver, gallstone, disk hernia, rheumatoid arthritis, epilepsia, thyroid-gland-related diseases, angina, cardiac dysrythmia, tuberculosis, bronchial asthma, kidney diseases and other diseases. Similarly, psychiatric illness was defined as having been diagnosed with and/or being currently under treatment of any psychiatric diseases. Regarding cigarette smoking, the questionnaire inquired whether the participants were nonsmokers, ex-smokers or current smokers. Nonsmokers and ex-smokers were later combined for statistical analyses. For alcohol consumption, participants were asked whether they were nondrinkers or current drinkers. For current drinkers, the frequency of drinking and the amount of alcohol consumed per session was assessed in terms of *go* (one go contains ~ 23 g of ethanol). A yes/no question was used to assess regular physical activity.

Statistical analyses

To explore gender differences, all data analyses were conducted separately in men and women. Characteristics of participants are presented as numbers (percentages) for categorical variables and mean with s.d. for continuous variables. Statistical differences in characteristics and anthropometric measurements and according to sleep duration categories in men and women were assessed using χ^2 test or Fisher's exact test for categorical variables and *t*-test or one-way analysis of variance for continuous variables.

Analysis of covariance was used to estimate adjusted means of BMI, WC, VFA and SFA across categories of sleep duration (<5 h, 5 to <6 h, 6 to <7 h and $\geqslant 7$ h). In model 1, we adjusted for age (continuous), regular physical activity (yes or no), current smoking status (nonsmokers or smoker) and current alcohol drinking (nondrinkers or drinker). Because physical illness has been found to be a potential confounder in the association between sleep duration and obesity,[21] we included it in model 2 in addition to the covariates in model 1. An additional model (model 3) was constructed for VFA and SFA. In addition to the covariates in model 2, SFA was included in the model for VFA, and VFA was included in the model for SFA. Trend of the association was assessed by using multiple linear regression models with ordinal numbers of 0 to 3 assigned to the categories of sleep duration with adjustments for the same covariates included in each model of analysis of covariance. In addition, statistical tests for a gender interaction were performed by including pair-wise interaction terms (that is, BMI $\times$ gender, WC $\times$ gender, VFA $\times$ gender and SFA $\times$ gender) in the multiple regression models. Two-sided *P*-values of <0.05 were regarded as statistically significant. We used IBM SPSS Statistics version 19.0 (IBM Corporation, New York, NY, USA) for all the statistical analyses.

RESULTS

The study subjects included 5400 men and 642 women with an age range between 30 to 75 years (mean $=53.3$ years ± 10.0 years in men and mean $=58.2$ years ± 9.4 years in women). Regarding the average sleep duration, 5.4% of the total study population slept <5 h, 42.6% slept 5 to <6 h, 39.4% slept 6 to <7 h and 12.6% slept $\geqslant 7$ h per day. The proportion of subjects sleeping >6 h per day was significantly higher in men than in women (53.0% vs 47.5%). Men were also significantly more likely to be current cigarette smokers (33.9% vs 3.3%) and current alcohol drinkers (76.4% vs 21.2%). The mean value of BMI, WC and VFA was significantly higher in men (mean $=24.2\pm3.1$ kg m^{-2}, mean $=86.7\pm8.3$ cm and mean $=124.2\pm53.8$ cm^2, respectively) than in women (mean $=23.1\pm3.3$ kg m^{-2}; mean $=83.6\pm9.5$ cm; and mean $=82.8\pm45.1$ cm^2; respectively). However, the mean value of SFA was significantly lower in men (mean $=136.5\pm57.8$ cm^2) than in women (mean $=185.9\pm75.8$ cm^2). Men were

significantly less likely to be living with at least one physical illness compared with women (37.1% vs 47.8%).

Tables 1 and 2 show characteristics of subjects according to sleep duration categories in men and women, respectively. In men, sleep duration increased as age increased. Men with shorter sleep duration were more likely to be current cigarette smokers and to be living with at least one physical illness. However, men with shorter sleep duration were less likely to be current alcohol drinkers, and they were less likely to involve with regular physical activity compared with those with longer sleep duration. In men, mean values of BMI, WC and SFA significantly decreased as sleep duration increased. In women, no significant association was found between characteristics of subjects and sleeping duration.

Table 3 shows the adjusted mean values of anthropometric indexes of subjects according to sleep duration categories in men. After adjustment for age, regular physical activity, cigarette smoking and alcohol drinking in model 1, mean values of BMI, WC and SFA decreased significantly with increasing sleep duration (*P*-values for trend <0.001). Adjustment for physical illnesses (model 2) did not significantly change the explanatory power of the models. For subjects sleeping '<5 h', '5 to <6 h', '6 to <7 h' and '$\geqslant 7$ h' per day, mean values of BMI were $24.8 \pm 3.5\,kg\,m^{-2}$, $24.3 \pm 3.2\,kg\,m^{-2}$, $24.0 \pm 2.7\,kg\,m^{-2}$ and $23.8 \pm 2.6\,kg\,m^{-2}$, respectively (*P*-values for trend <0.001), and mean values of WC were 87.9 ± 9.3 cm, 86.9 ± 8.7 cm, 86.4 ± 7.8 cm and 85.7 ± 7.5 cm, respectively (*P*-values for trend <0.001). The significant inverse association between sleep duration and SFA was also not attenuated after additional adjustment for physical illnesses (model 2) and VFA (model 3). In fully adjusted model, the mean values of SFA for subjects sleeping '<5 h', '5 to <6 h', '6 to <7 h' and '$\geqslant 7$ h' per day were $145.8 \pm 67.4\,cm^2$, $138.7 \pm 61.5\,cm^2$, $134.7 \pm 60.4\,cm^2$ and $132.5 \pm 49.2\,cm^2$, respectively (*P*-values for trend <0.001). Sleep duration was not appreciably associated with VFA in men. As shown in Table 4, sleep duration was not significantly associated with BMI, WC, VFA or SFA in any models in women. Gender interaction tests were all statistically significant for all the outcomes of interest (all *P*-values <0.001).

DISCUSSION

In this cross-sectional study, we investigated the relationship of sleep duration with general obesity and abdominal fat areas. To the best of our knowledge, this is the first study of its kind in which CT scanner was used to measure WC, VFA and SFA. We found that short sleep duration was strongly associated with higher BMI, WC and SFA in men. The association was independent of the effects of potential confounding factors such as physical and psychiatric illnesses. However, sleep duration was not appreciably associated with VFA. Apparent gender differences were observed as significant relationship was not detected between sleep duration and any obesity-related measures in women.

Table 1. Characteristics of subjects according to sleep duration categories in men

Variables	*Average sleep duration (hours per day)*					
	Total	*<5*	*5 to <6*	*6 to <7*	*⩾7*	P-*value*
Number of subjects	5400	272	2285	2152	690	
Age (years, mean ± s.d.)	53.3 ± 10.0	49.1 ± 9.4	50.5 ± 9.2	54.5 ± 9.8	60.3 ± 9.6	<0.001
Current smokers (*n*, %)	1829 (33.9)	92 (33.8)	832 (36.4)	721 (33.5)	184 (26.7)	<0.001
Current alcohol drinkers (*n*, %)	4127 (76.4)	191 (70.2)	1735 (75.9)	1656 (77.0)	545 (79.0)	0.03
Regular physical activity (*n*, %)	2419 (44.8)	82 (30.1)	942 (41.2)	1028 (47.8)	379 (54.9)	<0.001
Physical illnesses[a] (*n*, %)	2001 (37.1)	88 (32.4)	743 (32.5)	826 (38.4)	344 (49.9)	<0.001
Body mass index (years, mean ± s.d.)	24.1 ± 3.0	24.9 ± 3.4	24.3 ± 3.2	24.0 ± 2.7	23.7 ± 2.5	<0.001
Waist circumference (cm, mean ± s.d.)	86.6 ± 8.3	88.1 ± 9.2	87.0 ± 8.7	86.3 ± 7.8	85.6 ± 7.4	<0.001
Visceral fat area (cm^2, mean ± s.d.)	124.1 ± 53.7	124.9 ± 54.6	122.2 ± 54.3	124.9 ± 52.6	127.2 ± 54.0	0.16
Subcutaneous fat area (cm^2, mean ± s.d.)	136.7 ± 57.2	152.5 ± 69.4	141.5 ± 61.8	133.4 ± 51.6	124.6 ± 48.5	<0.001

[a]Physical illness was defined as having and/or being currently under treatment for at least one of the following diseases: hypertension, diabetes mellitus, hyperlipidemia, hyperuricemia, anemia, gastric ulcer, duodenal ulcer, colon polyp, chronic hepatitis, fatty liver, gallstone, disk hernia, rheumatoid arthritis, epilepsia, thyroid-gland-related diseases, angina, cardiac dysrythmia, tuberculosis, bronchial asthma, kidney diseases and other diseases.

Table 2. Characteristics of subjects according to sleep duration categories in women

Variables	*Average sleep duration (hours per day)*					
	Total	*<5*	*5 to <6*	*6 to <7*	*⩾7*	P-*value*
Number of subjects	642	57	286	230	69	
Age (years, mean ± s.d.)	58.2 ± 9.4	57.5 ± 9.6	57.2 ± 9.2	59.2 ± 9.7	59.6 ± 8.9	0.04
Current smokers (*n*, %)	21 (3.3)	1 (1.8)	12 (4.2)	3 (1.3)	5 (7.2)	0.06
Current alcohol drinkers (*n*, %)	136 (21.2)	11 (19.3)	66 (23.1)	39 (17.0)	20 (29.0)	0.13
Regular physical activity (*n*, %)	295 (46.0)	27 (47.4)	120 (41.9)	118 (51.3)	32 (46.3)	0.20
Physical illnesses[a] (*n*, %)	307 (47.8)	31 (54.4)	126 (44.1)	118 (51.3)	32 (46.4)	0.29
Body mass index (years, mean ± s.d.)	23.1 ± 3.4	23.2 ± 3.0	23.3 ± 3.6	22.9 ± 3.2	23.0 ± 3.5	0.32
Waist circumference (cm, mean ± s.d.)	83.4 ± 9.5	83.2 ± 9.1	83.5 ± 9.8	83.2 ± 9.0	83.8 ± 9.5	0.83
Visceral fat area (cm^2, mean ± s.d.)	82.2 ± 44.7	88.1 ± 46.5	80.4 ± 44.8	82.8 ± 44.6	82.4 ± 42.8	0.98
Subcutaneous fat area (cm^2, mean ± s.d.)	185.9 ± 74.6	182.7 ± 66.7	186.0 ± 75.2	185.3 ± 73.8	190.5 ± 74.6	0.71

[a]Physical illness was defined as having and/or being currently under treatment for at least one of the following diseases: hypertension, diabetes mellitus, hyperlipidemia, hyperuricemia, anemia, gastric ulcer, duodenal ulcer, colon polyp, chronic hepatitis, fatty liver, gallstone, disk hernia, rheumatoid arthritis, epilepsia, thyroid-gland-related diseases, angina, cardiac dysrythmia, tuberculosis, bronchial asthma, kidney diseases and other diseases.

Table 3. Adjusted mean values of anthropometric indexes of subjects according to sleep duration categories in men

Variables	*Average sleep duration (hour per day)*				
	<5	*5 to <6*	*6 to <7*	*⩾7*	P *for trend*[a]
Number	272	2285	2152	690	
Body mass index (kg m^{-2})					
Model 1[b]	24.8 ± 3.5	24.3 ± 3.2	24.0 ± 2.7	23.8 ± 2.6	<0.001
Model 2[c]	24.8 ± 3.5	24.3 ± 3.2	24.0 ± 2.7	23.8 ± 2.6	<0.001
Waist circumference (cm)					
Model 1[b]	87.9 ± 9.3	86.9 ± 8.7	86.4 ± 7.8	85.8 ± 7.5	<0.001
Model 2[c]	87.9 ± 9.3	86.9 ± 8.7	86.4 ± 7.8	85.7 ± 7.5	<0.001
Visceral fat area (cm^2)					
Model 1[b]	127.4 ± 54.6	124.3 ± 54.4	124.0 ± 52.6	122.0 ± 54.0	0.23
Model 2[c]	127.3 ± 54.6	124.4 ± 54.4	124.1 ± 52.6	121.4 ± 53.9	0.16
Model 3[d]	121.1 ± 53.9	123.1 ± 53.8	125.2 ± 52.9	124.7 ± 54.4	0.09
Subcutaneous fat area (cm^2)					
Model 1[b]	147.9 ± 69.5	138.9 ± 61.8	134.7 ± 51.6	131.3 ± 48.6	<0.001
Model 2[c]	147.9 ± 69.5	139.0 ± 61.9	134.7 ± 51.6	130.8 ± 48.5	<0.001
Model 3[e]	145.8 ± 67.4	138.7 ± 61.5	134.7 ± 51.6	132.5 ± 49.2	<0.001

Values are means ± s.d. [a]*P* for trend values were based on linear regression analyses with ordinal numbers 0 to 3 assigned to lowest through highest categories of sleep duration. [b]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no) and current alcohol drinking (yes or no). [c]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no) and physical illnesses (yes or no). [d]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no), physical illnesses (yes or no) and subcutaneous fat area (continuous). [e]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no), physical illnesses (yes or no) and visceral fat area (continuous).

Table 4. Adjusted mean values of anthropometric indexes of subjects according to sleep duration categories in women

Variables	*Average sleep duration (hour per day)*				
	<5	*5 to <6*	*6 to <7*	*⩾7*	P *for trend*[a]
Number	57	286	230	69	
Body mass index (kg m^{-2})					
Model 1[b]	23.2 ± 3.0	23.3 ± 3.6	22.9 ± 3.2	23.0 ± 3.5	0.24
Model 2[c]	23.1 ± 2.9	23.3 ± 3.5	22.9 ± 3.2	23.0 ± 3.6	0.28
Waist circumference (cm)					
Model 1[b]	83.3 ± 9.1	83.6 ± 10.0	83.1 ± 9.0	83.5 ± 9.6	0.79
Model 2[c]	83.1 ± 8.9	83.7 ± 10.1	83.0 ± 8.9	83.6 ± 9.7	0.86
Visceral fat area (cm^2)					
Model 1[b]	89.0 ± 46.5	82.0 ± 44.9	81.1 ± 44.7	80.8 ± 42.9	0.34
Model 2[c]	88.3 ± 45.8	82.1 ± 45.1	81.0 ± 44.6	81.2 ± 43.2	0.38
Model 3[d]	89.7 ± 47.3	81.7 ± 44.4	81.6 ± 45.1	79.7 ± 42.2	0.17
Subcutaneous fat area (cm^2)					
Model 1[b]	183.3 ± 66.7	186.9 ± 75.3	184.3 ± 73.8	189.2 ± 82.1	0.88
Model 2[c]	182.0 ± 65.2	187.2 ± 76.4	184.1 ± 73.6	189.9 ± 82.6	0.81
Model 3[e]	174.9 ± 62.3	187.2 ± 76.5	185.4 ± 74.9	191.0 ± 84.2	0.29

Values are means ± s.d. [a]*P* for trend values were based on linear regression analyses with ordinal numbers 0 to 3 assigned to lowest through highest categories of sleep duration. [b]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no) and current alcohol drinking (yes or no). [c]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no) and physical illnesses (yes or no). [d]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no), physical illnesses (yes or no) and subcutaneous fat area (continuous). [e]Adjusted for age (continuous), regular physical activity (yes or no), current cigarette smoking (yes or no), current alcohol drinking (yes or no), physical illnesses (yes or no) and visceral fat area (continuous).

This study extends the understanding in the literature of sleep and obesity research in which sleep deprivation has been considered as a potential predictor of obesity, frequently defined by using BMI. BMI is not a valid proxy for body fat mass, and existing BMI cutoffs are not suitable for the classification of individuals as normal weight, overweight or obese in Asians.[26] Asians have proportionally more fat for a similar BMI level and are at increased cardiovascular risk at lower BMI levels as compared with Caucasians.[27] Findings from our study suggest that, in working Japanese men, short sleep duration is associated not only with

general obesity, but also with increased subcutaneous fat mass, supporting a role of chronic sleep restriction in obesity pathogenesis.

Reviews of several cross-sectional and prospective studies among child and adult populations around the world have found fairly uniform results that short sleep duration is associated with obesity[7] and weight gain.[16] However, in their most recent review of prospective studies, Nielsen *et al.*[2] concluded that short sleep duration is consistently associated with development of obesity in children and young adults, but the findings were less consistent in older adults. Furthermore, sleep duration was not associated with BMI in a population-based cohort study among Japanese aged 40 to 69 years,[17] as well as in a prospective multicenter cohort study among early-middle-aged adults (age range of 38 to 50 years) in the United States.[18] In a German study, the significant association between short sleep duration and BMI did not persist after controlling for physical health and emotional status.[21]

This study is the first in the field to formally assess physical and psychiatric illnesses and to assess the relationship between sleep duration and obesity independent of these factors. These confounders may lead to a relationship in the opposite direction; obesity predisposes to physical or psychiatric illnesses, which in turn cause reduced sleep duration. Previous studies have ignored this explanatory pathway or attempted to address it by using self-reported data obtained from a single question on the overall physical and mental health of the participants.[21] Such a measure is not sensitive and does not capture severity of the illnesses. In our analyses, we were not able to show a significant attenuation of the association between short sleep duration and obesity after excluding subjects with psychiatric illnesses and controlling for physical illnesses. These findings suggest that the association among our study population may not be explained by this pathway. Further studies are needed to investigate the possible confounding effects of physical and mental disorders on the relationship between sleep duration and obesity.

It is worth noting that short sleep duration did not show any significant association with general obesity and central abdominal fat areas among women in this study. Similar findings were also found in a study among a large Japanese working population in which no prospective association between sleep duration and obesity or weight gain was detected in women.[19] This finding is also consistent with results obtained from other studies in western populations.[28] In contrast, a study in Spain showed that the significant association between sleep duration and weight gain was observed in women, but not in men.[20] However, direct comparison with men might be made with caution as the mean age of women in our study was roughly 5 years older than that in men. In the Zurich Cohort Study, the relationship between sleep duration and weight weakened as participants aged.[29] Furthermore, our bivariate results show that sleep duration was not related to any obesity-related characteristics in women.

Based on experimental studies of sleep deprivation, a number of causal pathways linking short sleep duration with obesity have been suggested. One mechanism by which sleep deprivation might predispose to weight gain is by increasing caloric intake. In short-term trials, sleep restriction leads to reduction in circulating leptin, elevations in ghrelin, subjective hunger and preferences for calorie-dense, refined-carbohydrate foods,[30] which contribute to the development of obesity. Alternatively, some have argued that, in an environment where food is readily available, curtailed sleep may simply represent an increased opportunity to eat, especially if most of the wake-time is spent in sedentary activities such as watching television where snacking is common.[31] Chronic sleep deprivation clearly leads to feeling fatigue that may in turn lead to obesity-related behavior including decreased energy expenditure, irregular eating habit and low consumption of fruits and vegetables.[32] In addition, activation of inflammatory pathways by sleep restriction may also be implicated in the development of obesity.[33]

The strengths of this study include the large sample size of men, the use of CT scanner to measure central abdominal fat areas and the comprehensive assessments of important covariates. The relationship between sleep duration and obesity may vary in association with underlying risk factors such as insomnia and psychological disorders that are potential comorbidities of sleep deprivation and other severe medical conditions that might affect body composition. With a broad variety of data obtained from a standardized collection, we were able to exclude subjects with a history of psychiatric illnesses, insomnia, stroke, myocardial infarction, cancer and diabetes mellitus. In this way, we extended previous findings by systematically assessing the association between sleep duration and obesity independent from the effects of these potential confounding factors.

Several limitations should also be recognized. First, because of the cross-sectional design, a causal relationship cannot be definitively established. However, experimental studies have confirmed that sleep restriction can have metabolic effects that may be relevant to weight homeostasis.[31] Future studies should evaluate how changes in sleep duration are related to changes in weight and body fat composition over time. Second, daily sleep duration was self-reported, which is a continued limitation in sleep epidemiological studies. However, the Nurses' Health Study has shown a good validity for sleep duration measured by using a similar question against 1-week sleep diaries.[34] Third, long sleepers (>8 h) were not specifically separated from normal sleepers (7 to 8 h). As a result, we were unable to examine the relation between long sleep and obesity, as many studies have reported a U-shaped association.[12,35] Furthermore, information regarding sleep duration did not allow us to distinguish the real 'sleep duration' and 'time in bed'. Finally, although we excluded subjects with history of insomnia, no adjustment was made for other important sleep disorders such as obstructive sleep apnea, which is presumed to play an important role in both sleep disruption and obesity.[36] Future research should examine whether obstructive sleep apnea accounts for the gender differences in the association between sleep duration and adiposity as previous studies found that Asian men appear to have an increased risk of obstructive sleep apnea at lower BMI levels than observed in Caucasian men.[37]

In conclusion, our findings suggest that short sleep duration is associated not only with general obesity, but also with subcutaneous fat mass in Japanese working men. Further research is needed to further explicate the biological mechanisms behind this relationship and to see whether interventions addressing inadequate sleep or poor sleep quality could treat or prevent obesity by taking gender differences into consideration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Original Article
EPIDEMIOLOGY/GENETICS

Obesity

Adiponectin and Visceral Fat Associate with Cardiovascular Risk Factors

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Objective: To examine the combined effect of CT-measured visceral fat area (VFA) and adiponectin level against the clustering of metabolic risk factors.

Design and Methods: The subjects were 6,996 Japanese. The subjects were divided according to the combinations of VFA and adiponectin level quartiles and the odds ratio for multiple risk factors of metabolic syndrome were calculated (adjusted for age and lifestyle factors using logistic regression analyses). Group with the lowest VFA and the highest adiponectin level was used as a reference. The correlation between adiponectin level and each metabolic risk factor was evaluated.

Results: The strongest correlation was observed between adiponectin level and high-density lipoprotein cholesterol levels ($r = 0.369$ and 0.439 for men and women). Both VFA and adiponectin level were independently associated with the clustering of metabolic risk factors (interaction $P = 0.58$ and 0.11 for men and women). The odds ratio for the clustering of metabolic risk factors in the group with the highest VFA and the lowest adiponectin level, compared with the group with the lowest VFA and the highest adiponectin level, was 12.7 (9.7-16.6) for men and 13.5 (6.0-30.2) for women.

Conclusion: The ability to detect metabolic syndrome could be improved by examining adiponectin level in conjunction with VFA.

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Introduction

The prevalence of metabolic syndrome has been growing globally with clusters of obesity, high blood pressure, impaired lipid metabolism, and hyperglycemia. Individuals with metabolic syndrome have a higher risk of cardiovascular disease and a subsequent increase in disease mortality or morbidity (1-3). Several criteria for the diagnosis of metabolic syndrome are used worldwide. The visceral adipose tissue is regarded as an endocrine organ, partly because it secretes adipocytokines and other vasoactive substances that can influence the risk of developing traits of metabolic syndrome (4). We recently demonstrated that measuring the visceral fat area (VFA) is superior in predicting the accumulation of multiple risk factors, compared with the subcutaneous fat area (SFA), BMI, and waist circumference (WC) measurements (5). Regarding the multiple risk factors of metabolic syndrome, the odds ratios for the VFA quintiles were 1.0 (ref.), 2.4, 3.4, 5.0, and 9.7 for men and 1.0 (ref.), 1.5, 2.6, 4.6, and 10.0 for women ($P < 0.001$ for trends in both sexes) (5).

Adiponectin is predominantly secreted by adipocytes, and the adiponectin level is reduced in individuals with obesity, insulin resistance, and type 2 diabetes (6-10). Low plasma adiponectin levels have recently been shown to predict the risk of developing type 2 diabetes in humans (9,11). The adiponectin level is also inversely associated with other traditional cardiovascular risk factors, such as blood pressure, total and low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels (12,13), and is positively related to high-density lipoprotein (HDL) cholesterol levels (12,14).

Some previous studies reported the impact of adiponectin levels on metabolic syndrome and its components (15); however, the sample sizes were insufficient. In addition, the combined effect of the VFA and adiponectin level has not been examined in an epidemiological study. Thus, we have examined the combined effect of the VFA and adiponectin level on the clustering of metabolic risk factors.

Methods

Survey

Among the 17,606 employees of the same company and their spouses who underwent a health examination in Japan between 2008

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TABLE 1 Characteristics of the subjects

	Men		Women	
	Mean	(SD)	Mean	(SD)
N	6,221		775	
Age, years	52.8	(10.2)	57.4	(9.7)
Body mass index, kg/m^2	24.1	(3.0)	23.0	(3.4)
Visceral fat area, cm^2	121.7	(53.4)	81.6	(46.3)
Subcutaneous fat area, cm^2	133.6	(56.4)	182.8	(76.9)
Adiponectin, log μg/mL	0.83	(0.20)	1.05	(0.21)
High blood pressure, %	38.9		34.1	
High triglyceride, %	35.4		23.7	
Low HDL cholesterol, %	11.0		17.8	
Hyperglycemia, %	57.8		40.8	
Multiple risk factors of metabolic syndrome, %	45.5		34.2	

and 2009, we analyzed 6,996 subjects ranging in age from 25 to 75 years (6,221 men and 775 women) who had undergone a computed tomography (CT) examination and answered a questionnaire on lifestyle factors and current treatments for metabolic conditions (hyperlipidemia, hypertension, or diabetes). The VFA was measured using a CT scanner and was calculated using a software application (fatPointer; Hitachi Medico, Tokyo, Japan) according to a protocol described elsewhere (11). Briefly, single slice imaging at the umbilical level was performed using a CT machine (Redix turbo; Hitachi Medico) while the subject was in a supine position. The imaging conditions were 120 kV, 50 mA, using a 5 mm thick slice. Height, weight, and body fat were measured using an automated scale (BF-220; Tanita, Tokyo, Japan) with the patient wearing a light gown. The BMI was defined as the weight (kg) divided by the square of the height (m^2). A blood sample was collected from each subject after more than 12 hours of fasting. The glucose level was measured using the glucose oxidase enzyme-electrode method (A&T, Tokyo, Japan). TG and HDL cholesterol levels were measured using an enzymatic colorimetric method (Cholestest TG; Sekisui Medical, Tokyo, Japan) and a nonsettling enzymatic method (Cholestest NHDL; Sekisui Medical), respectively. Adiponectin levels were measured using an immunoturbidimetric method (Adiponectin Latex Kit for humans; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Blood pressure was measured using an automated sphygmomanometer (Kentaro ADVANCE BP-203RV III A/B; Colin, Tokyo, Japan). This study was approved by the ethics review committee of the National Center for Global Health and Medicine. Written informed consent was obtained from all the subjects.

Definition of the state of risk factor clustering

In this study, subjects were defined using the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines (6) published in 2005: [1] high TG (TG $\geq$ 150 mg/dL), [2] low HDL cholesterol (HDL cholesterol <40 mg/dL in men and <50 mg/dL in women), [3] high blood pressure (systolic blood pressure $\geq$130 mm Hg or diastolic blood pressure $\geq$85 mm Hg), [4] hyperglycemia (fasting glucose $\geq$100 mg/dL), and [5] multiple risk factors (having two or more of components [1-4] listed above). Subjects currently receiving treatment for hyperlipidemia, hypertension, or diabetes were deemed as having the respective risk factor, regardless of the biochemical value.

Statistical analyses

We calculated the Pearson's correlation coefficients between the adiponectin level and each metabolic risk factor. We divided the subjects according to quartiles of the adiponectin level and calculated the odds ratio for multiple risk factors of metabolic syndrome. We adjusted for age, smoking habits (never, past, current), alcohol consumption (nondrinker, drinker consuming less than 2 go per day [the go is a conventional unit of alcohol intake in Japan and contains approximately 23 g of ethanol], or drinker consuming more than 2 go per day), and regular fitness habit (based on a single yes/no question in the questionnaire) using a logistic regression analysis, with the highest adiponectin level group used as a reference. We calculated the odds ratio for the multiple risk factors of metabolic syndrome for a +1 SD increment in the quintile categories of VFA and a +1 SD increment in the quintile categories of adiponectin levels. Furthermore, we divided the subjects according to combinations of VFA and adiponectin level quartiles and calculated the odds ratio for multiple risk factors of metabolic syndrome adjusted for the above-mentioned variables, using the category with the lowest VFA and the highest adiponectin level as the reference. VFA, adiponectin levels, and their interaction term (VFA $\times$ adiponectin levels) were included as independent variables in the logistic regression model to examine the interaction effect between VFA and adiponectin levels on the risk of clustering of metabolic risk factors. The stepwise procedure was used to select variables in the multiple logistic regression model with $P < 0.1$ for entry and $P < 0.05$ for removal. All the analyses were performed using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago. IL, USA).

Results

The characteristics of the subjects are shown in Table 1. The mean (SD) age of the subjects was 52.8 $\pm$ 10.2 years for men and 57.4 $\pm$ 9.7 years for women. The mean VFA was 121.7 $\pm$ 53.4 cm^2 in men and 81.6 $\pm$ 46.3 cm^2 in women. The mean BMI was 24.1 $\pm$ 3.0 kg/m^2 in men and 23.0 $\pm$ 3.4 kg/m^2 in women. The mean log adiponectin level was 0.83 $\pm$ 0.20 μg/mL in men and 1.05 $\pm$ 0.21 μg/mL in women. The prevalence of multiple risk factors of metabolic syndrome was 45.5% in men and 34.2% in women.

Table 2 shows the partial correlations between adiponectin level and each metabolic risk factor. The HDL cholesterol level positively correlated with adiponectin level ($P < 0.001$). Other metabolic risk factors negatively correlated with the adiponectin level ($P < 0.001$).

The odds ratios for each component of metabolic syndrome according to the adiponectin level are shown in Figure 1. The odds ratios for a high TG level, a low HDL cholesterol level, high blood pressure, and hyperglycemia decreased with increasing quartile categories of adiponectin levels. For the multiple risk factors of metabolic syndrome, the odds ratios (95% confidence intervals [CI]) of the Q1, Q2, Q3, and Q4 adiponectin level categories were 3.4 (3.0-4.0), 2.1 (1.8-2.5), 1.5 (1.3-1.7), and 1.0 (ref.) for men and 4.3 (2.7-6.9), 2.5 (1.6-4.1), 1.5 (0.9-2.4), and 1.0 (ref.) for women. The odds ratio (95% CI) of the lowest (Q1) adiponectin level category

TABLE 2 Partial correlation between adiponectin level and each metabolic risk factor

	Men	Women
VFA	−0.364	−0.428
SFA	−0.220	−0.234
BMI	−0.281	−0.237
log TG	−0.340	−0.342
HT	−0.104	−0.153
HDL cholesterol	0.369	0.439
FG	−0.103	−0.177

VFA, visceral fat area; SFA, subcutaneous fat area; BMI, body mass index; TG, triglyceride; HT, hypertension; FG, fasting glucose.
P-values are all less than 0.001.
Values are partial correlation coefficients adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

for a high TG level was 3.9 (3.4-4.6) in men and that for a low HDL cholesterol level was 5.8 (4.4-7.8) in men and 9.9 (4.8-20.1) in women.

In Table 3, the odds ratios of adiponectin and VFA levels for the clustering of multiple risk factors of metabolic syndrome are shown according to the VFA and adiponectin quartiles, respectively. For men, the increased adiponectin levels were significantly related to reduced clustering of metabolic risk factors, regardless of the VFA category ($P = 0.58$ for interaction VFA × adiponectin). For women, however, the odds ratio for a +1 SD in the VFA slightly weakened according to the increment of adiponectin level category ($P = 0.11$ for interaction of VFA × adiponectin). The odds ratio for multiple risk factors of metabolic syndrome according to combined groups of VFA and adiponectin are depicted in Figure 2. The odds ratio for the multiple risk factors of metabolic syndrome in the category with the highest VFA and the lowest adiponectin levels were 12.7 (9.7-16.6) for men and 13.5 (6.0-30.2) for women. We conducted stepwise logistic regression analyses among the largest quartile group of VFA, with hypoadiponectinemia (lowest vs. highest quartile group) as the independent variable. The result revealed that smoking, high TG, low HDL cholesterol, and older age were associated with hypoadiponectinemia in men. Low HDL cholesterol and age were associated with hypoadiponectinemia in women (data not shown).

Discussion

We observed a strong association between the combined effect of an increasing VFA and a decreasing adiponectin level and the prevalence of multiple risk factors of metabolic syndrome. Both the VFA and the adiponectin level were independently associated with the clustering of metabolic risk factors. Among the components of metabolic syndrome, the adiponectin level had a particularly strong impact on a high TG level in men and a low HDL cholesterol level in both men and women.

Only one previous report, studying 68 obese Korean subjects, demonstrated an association between the adiponectin level and VFA. The adiponectin level was inversely correlated with the VFA ($r = -0.691$, $P = 0.009$ in men, $r = -0.319$, $P = 0.002$ in women). Levels of a high molecular weight adiponectin also negatively correlated with the VFA ($r = -0.650$, $P = 0.016$ in men, $r = -0.370$, $P = 0.005$ in women) but not with the BMI or SFA, suggesting hypoadiponectinemia may represent a dysfunction of adipose tissue during obesity (16).

It was unknown whether adiponectin levels were correlated with disease, even when the VFAs were the same. Therefore, we compared the prevalence of multiple risk factors of metabolic syndrome with combinations of the VFA and adiponectin level. We found a markedly increased risk of clustering of metabolic syndrome among individuals who had a low adiponectin level and a high VFA. It was revealed that even when VFAs were the same, hypoadiponectinemia was associated with older age, smoking, and lipid metabolism (high TG and low HDL cholesterol) in men. In women, hypoadiponectinemia was associated with older age and low HDL cholesterol. Thus, it was confirmed that adiponectin correlated with lipid metabolism independent of VFA; from this, we concluded that adiponectin correlated with the clustering of metabolic risk factors. However, even

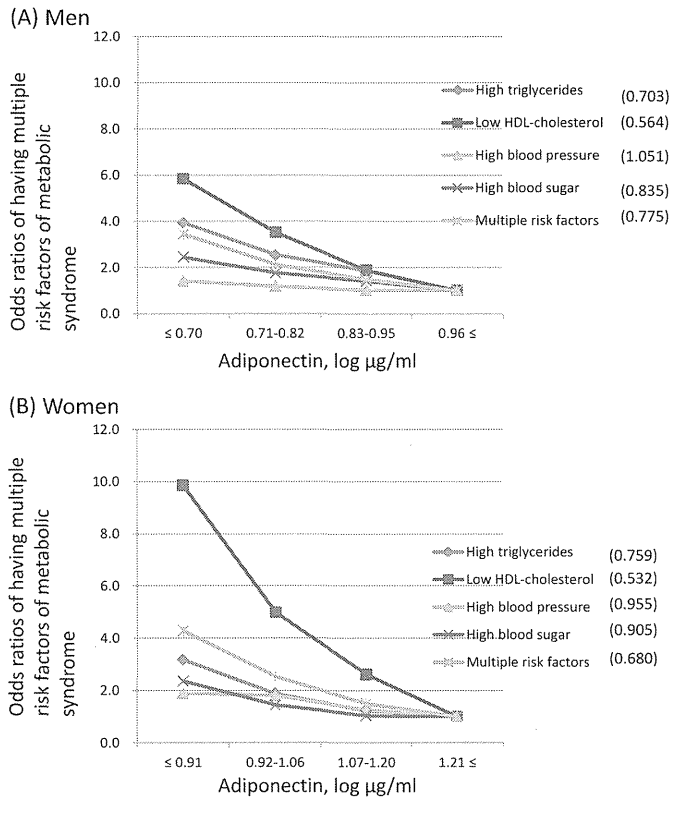

FIGURE 1 Odds ratios for clustering of metabolic risk factors according to the quartiles of adiponectin. The definition of metabolic risk factors is based on the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines published in 2005. Subjects with two or more of the metabolic risk factors (except for waist circumference) were defined as having multiple risk factors. Odds ratios for clustering of metabolic risk factors according to the quartiles (Q1-Q4) of adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no). Values in the case arcs are odds ratios for +1 SD increments of adiponectin. (Multiple risk factors; multiple risk factors of metabolic syndrome.)

TABLE 3 Odds ratios for clustering of metabolic risk factors

		n		Odds ratios of +1 SD increment of adiponectin	
Men					
VFA (cm^2)	≤84.80	1,557	Q1	0.8	(0.7-0.9)
	84.81-120.10	1,559	Q2	0.8	(0.7-0.9)
	120.11-156.90	1,553	Q3	0.8	(0.7-0.9)
	156.91≤	1,552	Q4	0.8	(0.7-0.9)
Women					
VFA (cm^2)	≤44.30	194	Q1	0.7	(0.4-1.2)
	44.31-77.90	195	Q2	0.5	(0.3-0.9)
	77.91-113.10	193	Q3	0.8	(0.6-1.2)
	113.11≤	193	Q4	0.6	(0.4-0.9)
		n		Odds ratios of +1 SD increment of VFA	
Men					
Adiponectin (log μg/mL)	≤0.70	1,620	Q1	2.1	
	0.71-0.82	1,556	Q2	2.2	(1.8-2.3)
	0.83-0.95	1,518	Q3	1.9	(1.9-2.5)
	≤0.96	1,527	Q4	2.1	(1.7-2.2)
Women					(1.9-2.4)
Adiponectin (log μg/mL)	≤0.91	198	Q1	2.6	(1.7-3.9)
	0.92-1.06	191	Q2	2.5	(1.6-3.8)
	1.07-1.20	193	Q3	2.1	(1.3-3.3)
	1.21≤	193	Q4	1.7	(1.1-2.5)

VFA, visceral fat area.
Odds ratios of multiple risk factors of metabolic syndrome according to 1 SD increment of VFA or adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

if the adiponectin levels were the same, weight gain led to a worsening of metabolic risk factors, including lipid. In the largest VFA group, adiponectin itself was related to lipid metabolism and smoking status. Therefore, it was shown that the ability to detect metabolic syndrome would be improved by examining the adiponectin level in conjunction with the VFA, as adiponectin correlated with metabolic risk factors independent of VFA and the correlation was most pronounced between lipid metabolism.

In a case–control study, high plasma adiponectin levels were associated with a lower risk of myocardial infarction (MI) over a follow-up period of 6 years among men without previous cardiovascular disease. After adjustment for matched variables, participants in the highest quintile, compared with the lowest quintile, of adiponectin levels had a significantly decreased risk of MI (relative risk [RR], 0.39; 95% confidence interval [CI], 0.23-0.64; P for trend <0.001). Further adjustment for the hemoglobin A1c or C-reactive protein levels had little impact, but additional adjustment for LDL and HDL cholesterol levels modestly attenuated this association (RR, 0.56; 95% CI, 0.32-0.99; P for trend =0.02) (17). A multiple logistic regression analysis revealed that hypoadiponectinemia was significantly and independently correlated with coronary artery disease (CAD) ($P < 0.0088$) among 450 Japanese men. The multivariate-adjusted odds ratios for CAD in the first, second, third, and fourth quartiles (95% confidence) were 2.051 (1.288-4.951), 1.221 (0.684-2.186), 0.749 (0.392-1.418), and 1.000, respectively (18). Furthermore, another study showed that BMI, serum TG concentration, and the presence of diabetes or CAD remained significantly related to the plasma adiponectin concentration. Weight reduction significantly elevated the plasma adiponectin levels in diabetic obese Japanese subjects (six men and seven women) and nondiabetic obese Japanese subjects (six men and three women). However, the sample size was very small in this study (6).

This study has several strengths and limitations. As one of its strengths, we directly assessed abdominal fat accumulation using CT scanning. This allowed the role of fat deposition in the development of metabolic syndrome and its components to be examined more closely. Secondly, the sample size of our study was sufficiently large (almost 7,000 subjects), and both sexes were included. Thirdly, we adjusted for alcohol consumption and physical activity, which may confound the association between abdominal fat accumulation and metabolic risk factors. However, the study was limited because of its cross-sectional design, and changes in the metabolic risk profile were not monitored.

In this study, we demonstrated a strong association between the combined effect of an increasing VFA and a decreasing adiponectin level with multiple risk factors of metabolic syndrome. Both the VFA and the adiponectin level were independently associated with the clustering of metabolic risk factors in a large Japanese population, which has a relatively low BMI compared with other

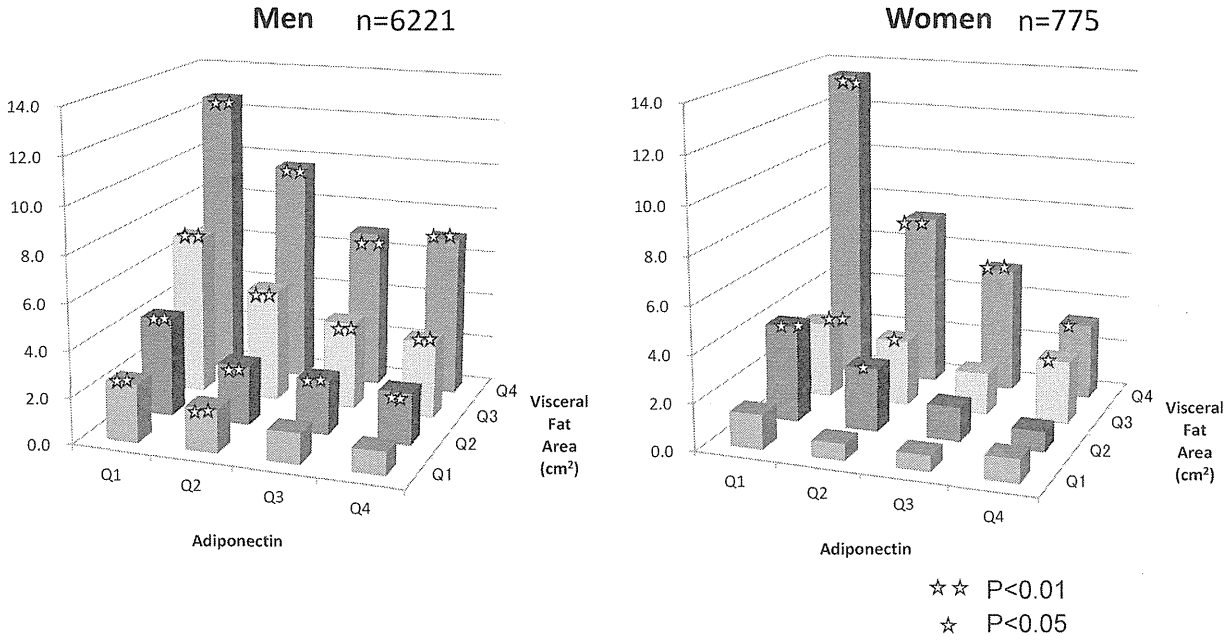

FIGURE 2 Odds ratios for multiple risk factors of metabolic syndrome according to combined groups of VFA and adiponectin. The definition of metabolic risk factors is based on the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines published in 2005. Subjects with two or more of the metabolic risk factors (except for waist circumference) were defined as having multiple risk factors. Odds ratios for multiple risk factors of metabolic syndrome are shown according to combined groups of VFA and adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

ethnicities. The present findings have important implications for the prevention of metabolic syndrome. Further prospective studies are needed to assess the impact of the VFA and adiponectin level on the incidence of metabolic syndrome or cardiovascular diseases.

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Effectiveness of Gefitinib against Non–Small-Cell Lung Cancer with the Uncommon EGFR Mutations G719X and L861Q

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Introduction: In non–small-cell lung cancer, an exon 19 deletion and an L858R point mutation in the epidermal growth factor receptor (EGFR) are predictors of a response to EGFR-tyrosine kinase inhibitors. However, it is uncertain whether other uncommon EGFR mutations are associated with sensitivity to EGFR-tyrosine kinase inhibitors.

Methods: A post-hoc analysis to assess prognostic factors was performed with the use of patients with EGFR mutations (exon 19 deletion, L858R, G719X, and L861Q) who were treated with gefitinib in the NEJ002 study, which compared gefitinib with carboplatin-paclitaxel as the first-line therapy.

Results: In the NEJ002 study, 225 patients with EGFR mutations received gefitinib at any treatment line. The Cox proportional hazards model indicated that performance status, response to chemotherapy, response to gefitinib, and mutation types were significant prognostic factors. Overall survival (OS) was significantly shorter among patients with uncommon EGFR mutations (G719X or L861Q) compared with OS of those with common EGFR mutations (12 versus 28.4 months; $p = 0.002$). In the gefitinib group (n = 114), patients with uncommon EGFR mutations had a significantly shorter OS (11.9 versus 29.3 months; $p < 0.001$). By contrast, OS was similar between patients with uncommon mutations and those with common mutations in the carboplatin-paclitaxel group (n = 111; 22.8 versus 28 months; $p = 0.358$).

Conclusions: The post-hoc analyses clearly demonstrated shorter survival for gefitinib-treated patients with uncommon EGFR mutations compared with the survival of those with common mutations and suggest that the first-line chemotherapy may be relatively effective for non–small-cell lung cancer with uncommon EGFR mutations.

Key Words: Gefitinib, G719X, L861Q, NEJ002, Uncommon epidermal growth factor receptor mutations.

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The clinical efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, has been demonstrated in non–small-cell lung cancer (NSCLC) patients in whom standard chemotherapy has failed.[1,2] Further studies have revealed that the presence of activating mutations in the EGFR kinase domain is strongly associated with the therapeutic efficacy of EGFR-TKIs.[3,4]

Randomized phase 3 trials have demonstrated that EGFR-TKIs significantly improve median progression-free survival (PFS) compared with platinum-doublet therapy in EGFR-mutated patients.[5–8] However, not all mutations in the EGFR kinase domain are responsive to EGFR-TKI treatment. These phase 3 trials have shown that EGFR-TKIs are effective for patients with common EGFR mutations, such as an exon 19 deletion or the L858R point mutation, which account for more than 90% of EGFR mutations. Retrospective studies and case reports suggest that some uncommon mutations are associated with sensitivity to EGFR-TKIs.[9–20] These mutations

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include G719X in exon 18, which accounts for approximately 3% of EGFR mutations, and L861Q in exon 21, which represents approximately 2% of EGFR mutations. However, these uncommon EGFR mutations have not been clearly shown to be predictive markers for the efficacy of EGFR-TKIs because of their low frequency.

To investigate the efficacy of gefitinib in patients with uncommon mutations, we conducted a post-hoc analysis of the NEJ002, which compared gefitinib and carboplatin-paclitaxel as first-line therapies for advanced NSCLC with activating EGFR mutations.

PATIENTS AND METHODS

Patient Population

We retrospectively analyzed the data of 225 patients who received gefitinib treatment at any point in the NEJ002 study.[6] The eligibility criteria of the NEJ002 study included the presence of advanced NSCLC harboring an EGFR mutation (exon 19 deletion or L858R, G719X, or L861Q point mutation) without the resistant EGFR mutation T790M (identified using the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method), no history of chemotherapy, an age of 75 years or younger, a performance status of 0 to 1, and appropriate organ function.[21,22] Patients provided a written informed consent. The study was conducted in accordance with the Helsinki Declaration of the World Medical Association. The protocol was approved by the institutional review board of each participating institution.

Treatment

Eligible patients were randomly assigned to receive either gefitinib (250 mg/day) or paclitaxel (200 mg/m^2)/carboplatin (area under the curve, 6.0) on day 1 every 3 weeks. Chemotherapy was continued for at least three cycles. Gefitinib was administered until the disease progressed, intolerable toxicities developed, or consent was withdrawn. The protocol recommended that the crossover regimen be used as a second-line treatment.

Clinical Assessments

The antitumor response to treatment was assessed using computed tomography every 2 months. Unidirectional measurements were adopted on the basis of the Response Evaluation Criteria in Solid Tumors (version 1.0).[23] PFS was evaluated from the date of randomization to the date when disease progression was first observed or death occurred. The treatment response and PFS were determined by an external review of computed tomography scans by experts who were not aware of the treatment assignments. Overall survival (OS) was evaluated from the date of randomization to the date of death.

Statistical Analysis

To assess prognostic factors for OS, we used univariate and multivariate Cox proportional hazards models. Kaplan–Meier survival curves were constructed for PFS and OS, and differences between groups were identified using the log-rank test. Differences in response rates were identified using Fisher's exact test. Each analysis was two sided, with a 5% significance level and a 95% confidence interval. All analyses were performed using SAS for Windows software (release 9.1; SAS Institute, Cary, NC).

RESULTS

Patient Population

A total of 230 chemonaive patients were enrolled in the NEJ002 study: 115 patients were assigned to receive gefitinib and 115 were assigned to receive carboplatin-paclitaxel (Fig. 1). To evaluate the efficacy of gefitinib in NSCLC patients with uncommon EGFR mutations, we analyzed the data of 114 patients in the gefitinib group and 111 patients in the carboplatin-paclitaxel group. We identified five patients who had uncommon EGFR mutations in each group. Two patients, who had common mutations and were treated with first-line chemotherapy consisting of carboplatin-paclitaxel, were excluded from the PFS analysis in the NEJ002 study. However, both were treated with gefitinib and were included in this post-hoc analysis. The demographic and disease characteristics of the patients with uncommon EGFR mutations were similar to those of patients with common EGFR mutations (Table 1). The characteristics of each patient with uncommon EGFR mutations are shown in supplementary Table S1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A494).

Survival Factors

In the univariate analysis of 225 patients who received gefitinib at any point, uncommon EGFR mutations had a significant detrimental effect on survival (Table 2). We also identified performance statuses 1 and 2, distant metastasis, brain metastasis, stable disease, and progressive disease as significant predictors of worse prognosis for standard chemotherapy and stable disease and progressive disease as significant predictors of worse prognosis for gefitinib. When these variables were included in the Cox proportional hazards model, we found that uncommon EGFR mutations, performance statuses 1 and 2, stable disease and progressive disease for standard chemotherapy, and stable disease and progressive disease for gefitinib had significant hazard ratios (Table 2).

Uncommon EGFR Mutations and Survival

The Kaplan–Meier curve for OS for uncommon versus common EGFR mutations is shown in Figure 2*A*. The OS was significantly shorter among patients with uncommon EGFR mutations compared with OS of those with common EGFR mutations in the overall population (12 versus 28.4 months; $p = 0.002$). A significantly shorter survival time was observed in patients with uncommon EGFR mutations compared with survival time in those with common EGFR mutations in the gefitinib group (11.9 versus 29.3 months; $p < 0.001$) (Fig. 2*B*). However, a similar survival time was observed between the subgroups of uncommon and common EGFR mutations in the carboplatin-paclitaxel group (22.8 versus 28 months; $p = 0.358$) (Fig. 2*C*).

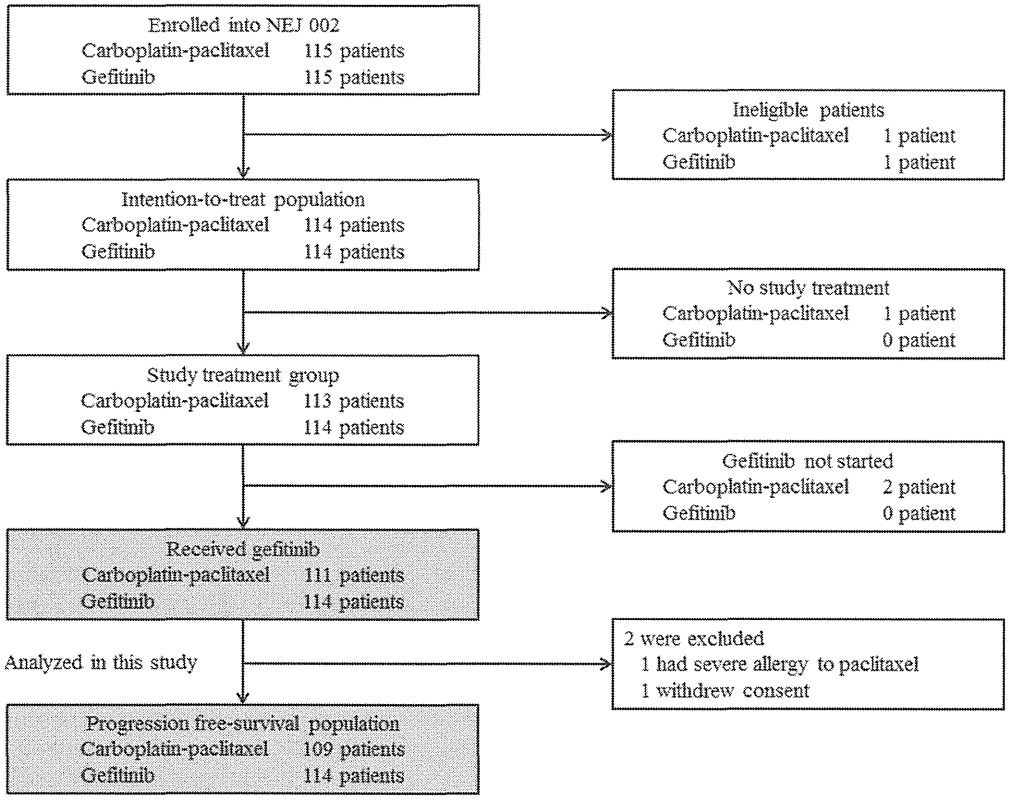

FIGURE 1. Enrollment, randomization, and follow-up of the study patients.

To examine whether the sequence of platinum doublet and gefitinib affected OS, we performed a further subgroup analysis. The survival time tended to be shorter among patients receiving first-line gefitinib compared with the survival time among those receiving first-line carboplatin-paclitaxel in the uncommon EGFR mutation group (11.9 versus 22.8 months; $p = 0.102$). Consistent with previous publications, a similar survival time was observed between patients receiving first-line gefitinib and those receiving first-line carboplatin-paclitaxel in the common EGFR mutation group (29.3 versus 28 months; $p = 0.378$).

Uncommon EGFR Mutations, PFS, and Response

In the gefitinib group, the median PFS was significantly shorter for patients with uncommon EGFR mutations compared with median PFS of those with common EGFR mutations (2.2 versus 11.4 months; $p < 0.001$) (Fig. 3*A*). By contrast, the median PFS did not differ significantly between patients with uncommon EGFR mutations and those with common EGFR mutations in the carboplatin-paclitaxel group (5.9 versus 5.4 months; $p = 0.847$) (Fig. 3*B*). The objective response rate was significantly lower in patients with uncommon EGFR mutations compared with the objective response rate in those with common EGFR mutations when treated with gefitinib (20% versus 76%; $p = 0.017$) (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). By contrast, similar objective response rates were observed for patients with uncommon EGFR mutations and those with common EGFR mutations in the carboplatin-paclitaxel group (20% versus 32%; $p = 0.336$) (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494).

DISCUSSION

Recent studies suggest that NSCLC patients with uncommon EGFR mutations are less responsive to EGFR-TKIs compared with patients with L858R and exon 19 deletions.[9–20] However, the efficacy of EGFR-TKIs in NSCLC patients with uncommon mutations has not been fully elucidated.

We conducted a post-hoc analysis of the NEJ002 study to evaluate the effectiveness of gefitinib against NSCLC with G719X or L861Q. The NEJ002 study, comparing gefitinib and standard carboplatin-paclitaxel chemotherapy as the first-line treatment for patients with EGFR mutations, demonstrated no significant difference in OS between gefitinib and carboplatin-paclitaxel.[6] In contrast to other phase 3 trials investigating EGFR-TKIs for patients with common EGFR mutations of exon 19 deletion and L858R, the NEJ002 is the only study that included uncommon EGFR mutations of G719X and L861Q.

The current study clearly demonstrated that NSCLC patients with the uncommon EGFR mutations G719X and L861Q had shorter survival than the survival of those with an exon 19 deletion or L858R mutation (Fig. 2). Our results are consistent with other clinical studies on EGFR-TKIs in

TABLE 1. Patient Characteristics

Number of Patients	Uncommon Mutation 10	Common Mutation 215
Sex		
Female	4	139
Male	6	76
Age (yr)		
Median	63	65
Range	42–75	35–75
Smoking status		
Never smoked	5	134
Smoker	5	81
Performance status		
0/1/2	5/5/0	105/107/3
Histology		
Adenocarcinoma	9	202
Others	1	13
Clinical stage		
Stage IIIB	3	32
Stage IV	6	165
Postoperative	1	18
Type of EGFR mutation		
G719X	7	
L861Q	3	
Exon 19 deletion		115
L858R		97
19 deletion + L858R		3

EGFR, epidermal growth factor receptor.

patients with uncommon EGFR mutations (supplementary Table S3, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). The overall response rate to EGFR-TKIs in patients with uncommon EGFR mutations was 41%, which is lower than the response rate to TKIs (62%–83%) of patients with common EGFR mutations.[7,8,24] In the NEJ002 study, G719X included G719C and G719S. No patients harbored G719A. To investigate the effectiveness of gefitinib on each uncommon EGFR mutations, we evaluated the difference in OS between patients with uncommon EGFR mutations (G719C versus G719S and G719X versus L861Q). There was no significant difference between these subgroups (data not shown).

This study showed that the PFS and OS tended to be shorter among patients treated with first-line gefitinib compared with PFS and OS among those treated with first-line carboplatin-paclitaxel in the uncommon EGFR mutation group (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). We also found poor disease control rate with gefitinib in patients with uncommon mutations. Three of five patients with uncommon mutations in the gefitinib group had progressive disease. By contrast, no patients with uncommon mutations had progressive disease in the carboplatin-paclitaxel group. Although the number of patients with uncommon mutations in each treatment group was small, platinum-doublet therapy might be a better choice than gefitinib for first-line therapy in patients with uncommon EGFR mutations. Because some of patients with uncommon mutations showed good clinical response to gefitinib in this study and they seemed to be heterogeneous in terms of response to gefitinib, administration of gefitinib should be considered for patients with uncommon mutations when disease progression was observed after first-line chemotherapy.

In vitro studies have indicated that the affinity of gefitinib for EGFR proteins with uncommon EGFR mutations is lower than the affinity of gefitinib for EGFR proteins with common EGFR mutations.[25] A sixfold or 14-fold higher concentration of gefitinib was required to inhibit the growth of cells expressing G719X or L861Q, respectively, compared with cells expressing L858R.[26] These results may explain the lack of response to gefitinib in patients with uncommon EGFR mutations. The authors also examined the sensitivity of G719X and L861Q mutations to erlotinib and irreversible TKIs.[27] Cells expressing G719X were less resistant to erlotinib than gefitinib in vitro; however, L861Q was resistant to both erlotinib and gefitinib. In contrast to erlotinib, irreversible TKIs inhibited the growth of cells with G719X or L861Q at a

TABLE 2. Univariate and Multivariate Analysis by Cox Proportional Hazards Model

	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (≥70/<70)	1.047	0.719–1.525	0.81			
Sex (female/male)	0.73	0.51–1.045	0.86			
Smoking status (+/−)	1.376	0.967–1.958	0.076			
Performance status (1, 2/0)	1.792	1.263–2.541	0.001	1.85	1.297–2.639	0.001
Histology (nonadeno/adeno)	0.647	0.302–1.387	0.263			
Types of EGFR-m (uncommon/common)	2.967	1.501–5.868	0.018	2.445	1.177–5.079	0.017
Distant metastasis (+/−)	4.914	1.113–5.741	0.027	2.849	1.241–6.54	0.135
Brain metastasis (+/−)	1.781	1.248–2.542	0.002	1.311	0.897–1.915	0.162
Response to Cb/TXL (SD, PD/CR, PR)	1.742	1.113–2.728	0.015	1.748	1.11–2.754	0.016
Response to G (SD, PD/CR, PR)	2.878	2.012–4.117	0.002	2.601	1.794–3.771	<0.001

HR, hazard ratio; CI, confidential interval; EGFR-m, epidermal growth factor receptor mutation; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Cb/TXL, carboplatin plus paclitaxel; G, gefitinib.

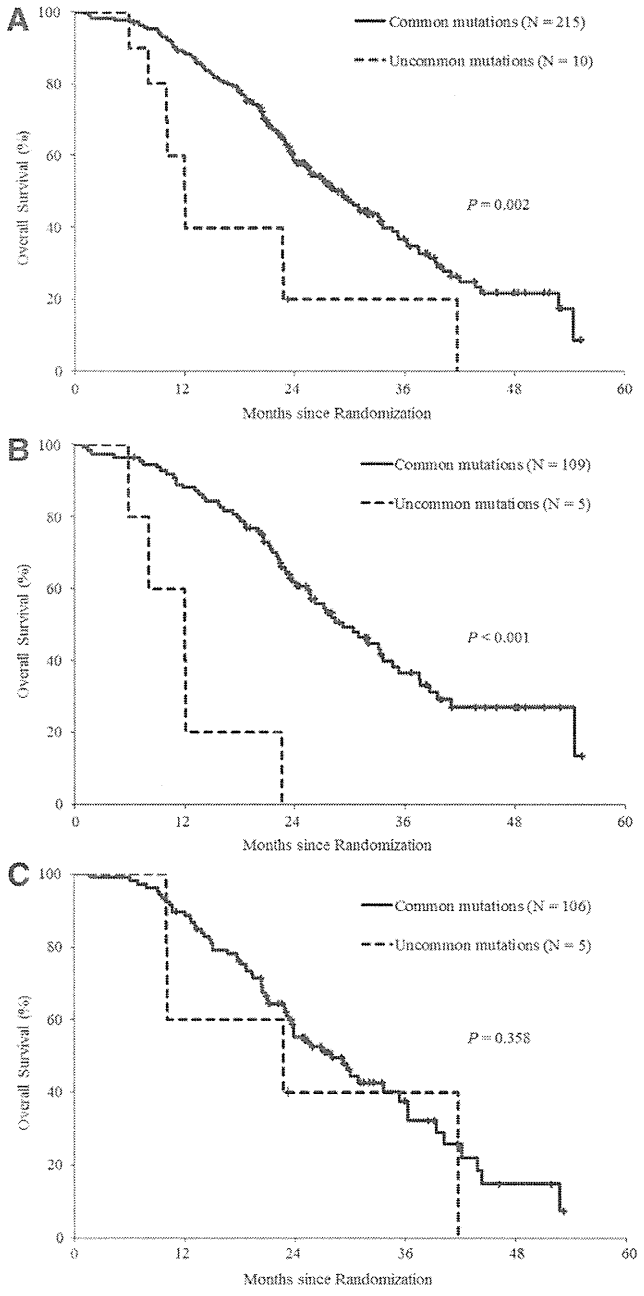

FIGURE 2. The overall survival curves of patients with common mutations and uncommon mutations in the entire population (*A*), the gefitinib group (*B*), and the carboplatin-paclitaxel group (*C*).

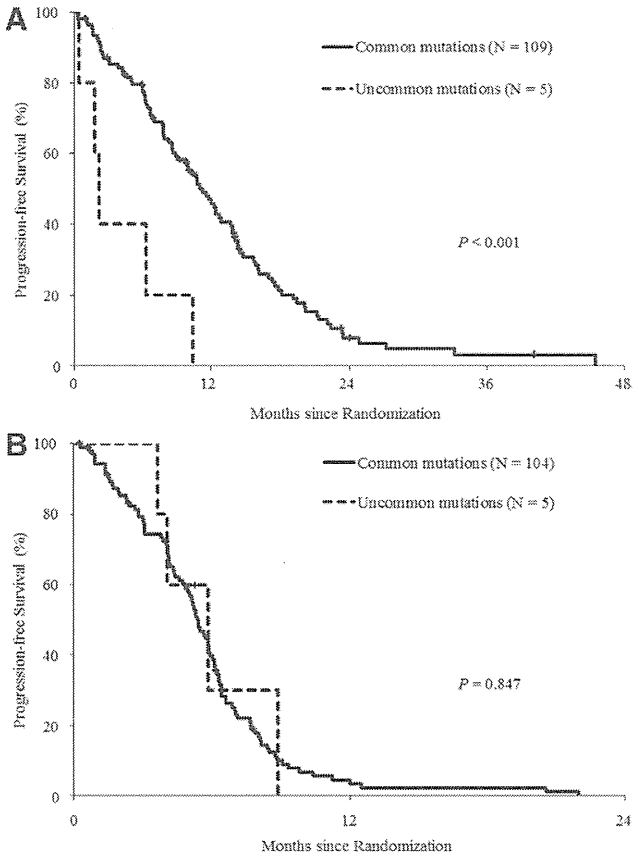

FIGURE 3. Progression-free survival curves in the gefitinib group (*A*) and the carboplatin-paclitaxel group (*B*) according to the type of epidermal growth factor receptor mutation.

lower concentration than those with wild-type EGFR. Indeed, Sequist et al.[28] reported that the effectiveness of an irreversible pan-ErbB receptor TKI, neratinib, on NSCLC patients with G719X. Niratinib induced partial responses in three of four patients with G719X and the fourth had durable stable disease for 40 weeks. It may be beneficial to evaluate erlotinib as a treatment for NSCLCs with G719X and irreversible EGFR-TKIs as treatments for NSCLCs with G719X and L861Q. Because previous phase 3 trials that investigated erlotinib or irreversible TKIs for NSCLC with EGFR mutations did not include uncommon EGFR mutations, further clinical studies may need to be performed.[7,8,29]

Another possible strategy for the treatment of uncommon EGFR mutations is the combination of EGFR-TKIs and cytotoxic agents. Our group has undertaken a randomized phase 3 trial to compare gefitinib plus carboplatin plus pemetrexed with gefitinib monotherapy for patients with NSCLC with an exon 19 deletion or an L858R, G719X, or L861Q EGFR mutation (NEJ009; University Hospital Medical Information Network Clinical Trials Registry [UMIN-CTR] number, UMIN000006340). The data from this study will advance the treatment of NSCLC with uncommon EGFR mutations.

In conclusion, our post-hoc analysis clearly demonstrated shorter survival of TKI-treated patients with uncommon EGFR mutations compared with survival of those with common EGFR mutations. Furthermore, the data suggest that the first-line chemotherapy may be relatively effective for NSCLC with uncommon EGFR mutations.

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BMC Cancer

CASE REPORT Open Access

Activity of EGFR-tyrosine kinase and ALK inhibitors for *EML4–ALK*-rearranged non–small–cell lung cancer harbored coexisting *EGFR* mutation

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Abstract

Background: The *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene represents a novel molecular target in a small subset of non–small–cell lung cancers (NSCLCs). The *EML4–ALK* fusion gene occurs generally in NSCLC without mutations in epidermal growth factor receptor *(EGFR)* and *KRAS*.

Case presentation: We report that a case of *EML4–ALK*-positive NSCLC with *EGFR* mutation had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

Conclusions: We described the first clinical report of a patient with *EML4–ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent EGFR-TKI and ALK inhibitor. *EML4–ALK* translocation may be associated with resistance to EGFR-TKI, and EGFR signaling may contribute to resistance to ALK inhibitor in *EML4–ALK*-positive NSCLC.

Keywords: EML4–ALK, *EGFR* mutation, Lung cancer

Background

The *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene was recently identified as a novel genetic alteration in non-small-cell lung cancer (NSCLC) [1]. *EML4–ALK* fusions have been detected in 2 to 7% of NSCLC patients. Patients harboring *ALK* rearrangements tend to be never and light smokers, have a history of adenocarcinoma, and be younger in age [1-6]. In general, the *EML4–ALK* fusion oncogene existed exclusively in NSCLC patients without the epidermal growth factor receptor (*EGFR*) gene mutation [1,7,8].

ALK inhibitors such as crizotinib are clinically effective in NSCLC patients harboring ALK rearrangements [9]. Crizotinib produced a high response rate and prolonged median progression-free survival among patients with ALK-positive NSCLC [9]. Crizotinib was recently approved by the US Food and Drug Administration and Japanese Ministry of Health, Labour and Welfare for the treatment of patients with advanced, ALK-rearranged NSCLC.

In this paper, we report a patient with NSCLC with concomitant ALK rearrangement and *EGFR* mutation that had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

Case presentation

In December 2009, a 55-year-old female who had never smoked was noted to have left lung opacity on a routine chest X-ray. No significant previous medical history was reported. Computed tomography (CT) scan of the chest revealed a 1.5 × 1.5 cm nodular lesion in the left upper lobe and hilar lymph node metastasis. Transthoracic needle biopsy histology revealed adenocarcinoma, and the histopathological subtype of the specimen was

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papillary adenocarcinoma with signet-ring cell components (Figure 1A-1C). The specimen was positive for periodic acid–Schiff (PAS) (Figure 1C). On immunohistochemical staining, the tumor cells were positive for thyroid transcription factor-1 (TTF-1) (Figure 1D). Laboratory findings were within normal range, except for the carcinoembryonic antigen (CEA) level of 158.0 ng/mL (normal range, 0 to 4.3 ng/mL) in the serum. She had multiple dorsal vertebra metastases (cT1N1M1b, stage IV).

Analysis for *EGFR* gene mutation was performed using a cytological specimen by means of the peptide nucleic acid–locked nucleic acid (PNA-LNA) polymerase-chain-reaction (PCR) clamp method as described previously [10,11]. The specimen showed a deletion in exon 19 (L747-A750del T751S). We collected mRNA from the same tumor specimens using Pinpoint Slide RNA Isolation System in order to clarify whether there was *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion gene in each tumor. Reverse transcription polymerase-chain-reaction (RT-PCR) followed by direct sequencing confirmed the presence of *EML4–ALK* variant 2 [1] (Figure 2). In addition, *EML4–ALK* was identified by using fluorescent in situ hybridization (FISH) for *ALK* rearrangements (Figure 3B) and was confirmed by immunohistochemistry for ALK expression in tumor [2] (Figure 3A).

A platinum doublet was chosen as first line therapy according to existing treatment protocol in 2009. Four cycles of combination chemotherapy comprising cisplatin and pemetrexed was administered at 3-week intervals. She was judged as having a stable disease. After 7 months, spinal magnetic resonance imaging (MRI) revealed progression of the dorsal vertebra lesions. Therefore, EGFR-TKI was chosen as a 2nd-line therapy. She received gefitinib therapy at 250 mg/day administered orally for 2 months. CT imaging of the chest showed that the pulmonary nodule was not growing after gefitinib therapy, and the tumor marker levels had not changed. However, spinal MRI demonstrated growing dorsal vertebra metastases 2 months after the start of gefitinib therapy. The carcinoembryonic antigen (CEA) level increased from 117 ng/ml to 250 ng/ml. Therefore, the patient was judged as having progressive disease. After local radiation therapy with a total of 30 Gy for dorsal metastases, a second EGFR-TKI was chosen given the stable primary disease. She received another EGFR-TKI, erlotinib (150 mg/day), as 3rd-line therapy. After being progression-free for 3 months, spinal MRI revealed a growing thoracic vertebra metastasis. She received 4th-line treatment with 2 cycles of docetaxel (DTX). However, her disease progressed 6 months later. Finally, she received a targeted inhibitor of ALK. The patient initially had SD associated with a temporary decrease in the CEA level from 743 ng/ml to 520 ng/ml, but her disease progressed after 4 months of therapy. The ALK inhibitor treatment was

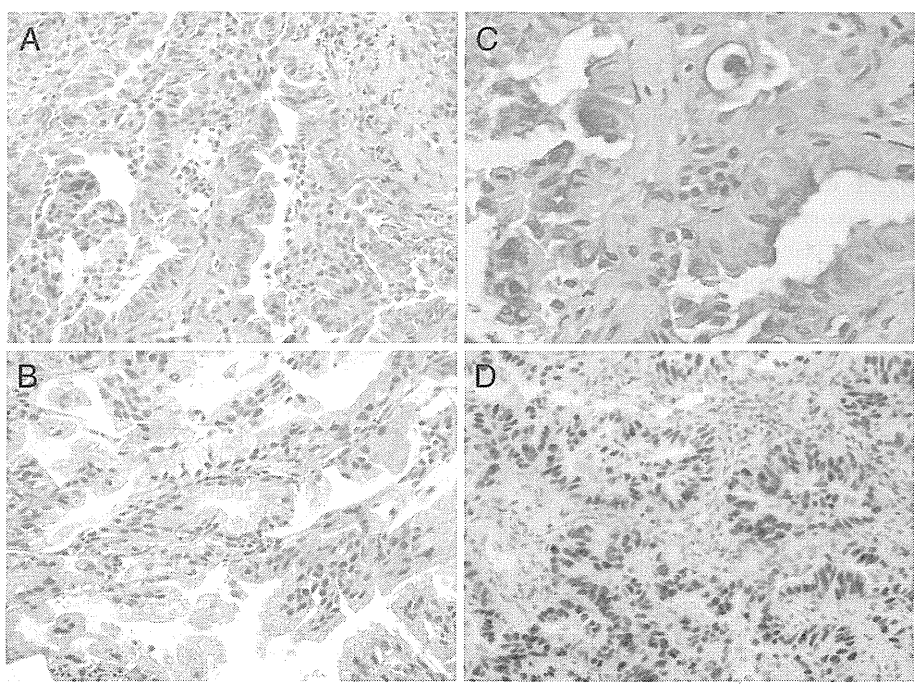

Figure 1 Histology of the primary tumour. (**A**) and (**B**) shows a papillary adenocarcinoma (hematoxylin and eosin 200× magnification), (**C**) a mucin stain shows positive for both signet-ring and papillary morphology (PAS, 400× magnification). **(D)** immunohistochemical analysis of lung adenocarcinoma specimens with *EML4-ALK* fusion using a monoclonal anti-TTF-1 antibody (200× magnification).

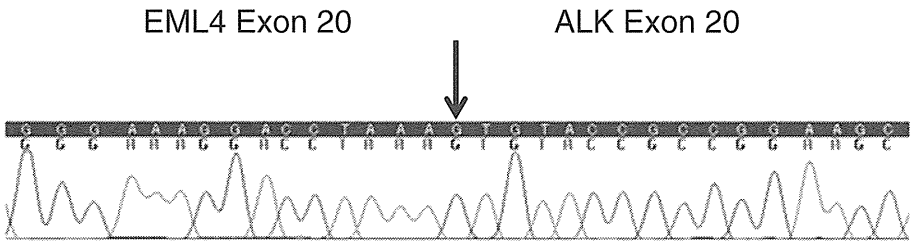

Figure 2 The sequence of the junction between *EML4* exon 20 and *ALK* exon 20.

ceased and full supportive care was given. All lines of therapy were well tolerated.

Discussion

We presented a patient with NSCLC with concomitant ALK rearrangement and *EGFR* mutation that had a response of stable disease to both EGFR-TKI and ALK inhibitors. The presence of *EML4–ALK* generally seems to be mutually exclusive of the presence of *EGFR* or *KRAS* mutations in NSCLC [1,7,8]. Previous reports showed twelve cases of *EML4–ALK*-positive lung cancer with *EGFR* mutation [3,12-17]. Only one patient with harboring *ALK* translocation and EGFR mutation was treated by ALK inhibitor has been reported [17]. Lee et al. reported two ALK-positive and EGFR-mutant NSCLC patient who did not respond to EGFR-TKI but achieved a durable partial response to ALK inhibitor [17]. The present patient was a woman with no history of smoking. Her pathological diagnosis was papillary adenocarcinoma with a signet-ring cell component, which was consistent with the previously reported characteristics of *EML4–ALK*-positive lung adenocarcinoma except for the *EGFR* mutation status [1-6]. It was reported that EGFR-TKI therapy among patients with advanced NSCLC and *EGFR* mutations revealed a response rate of more than 60% and progression-free survival of 9 to 14 months [11,18,19]. In addition, recent reports showed that ALK inhibition in NSCLC patients with the *ALK* rearrangement resulted in tumor shrinkage or stable disease in most patients [9]. Unfortunately, EGFR-TKI treatment was not effective in the tumor regression nor tumor marker level of present patient (disease might be controlled), but treatment with an ALK inhibitor resulted in SD with decreasing tumor markers. Therefore, this case showed that ALK rearrangement might be superior to EGFR mutation for the driver mutation.

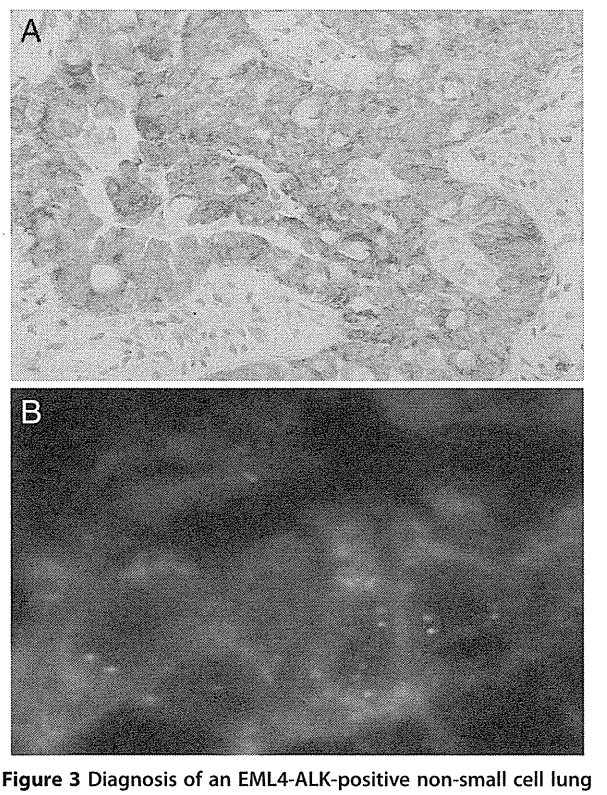

Figure 3 Diagnosis of an EML4-ALK-positive non-small cell lung cancer. (**A**) Immunostaining for ALK protein expression in tumor cells. (**B**) The results of a break-apart FISH assay of tumor cells from a patient with rearrangement of the gene encoding *ALK*.

It was reported that *EML4–ALK* fusion was associated with resistance to EGFR-TKIs [20]. Patients with NSCLC in the *EML4–ALK* cohort and the wild type cohort showed similar response rates to platinum-based combination chemotherapy and no difference in overall survival [20]. Whereas *EGFR* mutations confer sensitivity to EGFR-TKIs, *EML4–ALK* is strongly associated with resistance to EGFR-TKIs. In a previous case of concomitant *EGFR* mutation and *ALK* translocation, the patient presented the most durable response to an EGFR-TKI and was a case demonstrating no EML4–ALK expression by immunohistochemistry with an *EML4–ALK* rearrangement characterized by an isolated 3_ FISH signal [12]. Our patient presented a concurrent *EML4-ALK* rearrangement and ALK expression by immunohistochemistry; however, EGFR-TKI was not effective.

Among patients with both *EML4–ALK* rearrangement and *EGFR* mutation, *in vitro* studies showed that EGFR signaling can contribute to ALK inhibitor resistance in EML4–ALK NSCLC [14]. In addition, these findings suggested that a cancer cell line that harbors a concurrent

ALK rearrangement and an *EGFR* mutation would be expected to be resistant to both single agent ALK and EGFR inhibitors [14]. We suggest that the combination of both ALK and EGFR inhibitors as early-line treatment may represent an effective therapy for this subset of NSCLC patients.

Conclusions

This is the first clinical report of a patient with *EML4–ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent EGFR-TKI and ALK inhibitor. The *EML4–ALK* fusion gene defines a new molecular subset of NSCLCs with distinct clinical and pathologic features. NSCLCs with *ALK* rearrangement are highly sensitive to ALK inhibition. However, EGFR signaling may contribute to ALK inhibitor resistance in *EML4–ALK* NSCLC. Therefore, we suggest that this provides a translational opportunity whereby laboratory studies should be undertaken to understand the biological link between ALK rearrangement and *EGFR* mutation, with a view to establishing whether there is preclinical justification for using combination therapy for NSCLC with concomitant ALK rearrangement and *EGFR* mutation.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Abbreviations

EML4: Echinoderm microtubule-associated protein-like 4; ALK: Anaplastic lymphoma kinase; NSCLC: Non-small cell lung cancer; EGFR: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; CT: Computed tomography; PAS: periodic acid–Schiff; TTF-1: Thyroid transcription factor-1; PNA-LNA: Peptide nucleic acid–locked nucleic acid; PCR: Polymerase chain reaction technique; FISH: Fluorescent in situ hybridization; SD: Stable disease; MRI: Magnetic resonance imaging (MRI); CEA: Carcinoembryonic antigen; RT-PCR: Reverse transcription polymerase chain reaction.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AM prepared the manuscript and the literature search; RN and MS reviewed and edited the manuscript; HM and AG corrected and revised the manuscript; KS, KK, SK, YM, MS and TS treated and observed the patient; MK and ST performed the histopathological, immunohistochemical examinations; and AY, KH, KT, NY and YI reviewed the manuscript. All authors read and approved of the final manuscript.

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