Original Article

Association of Genetic Polymorphism with Taxane-induced Peripheral Neuropathy: Sub-analysis of a Randomized Phase II Study to Determine the Optimal Dose of 3-week Cycle Nab-Paclitaxel in Metastatic Breast Cancer Patients

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Chemotherapy-induced peripheral neuropathy (CIPN) is an important clinical challenge that threatens patients' quality of life. This sub-study of the ABROAD trial investigated the influence of single nucleotide polymorphisms (SNPs) on CIPN, using genotype data from a randomized study to determine the optimal dose of a 3-week-cycle regimen of nab-paclitaxel (q3w nab-PTX) in patients with metastatic breast cancer (MBC). Patients with HER2-negative MBC were randomly assigned to three doses of q3w nab-PTX (SD: 260 mg/m^2 vs. MD: 220 mg/m^2 vs. LD: 180 mg/m^2). Five SNPs (*EPHA4*-rs17348202, *EPHA5*-rs7349683, *EPHA6*-rs301927, *LIMK2*-rs5749248, and *XKR4*-rs4737264) were analyzed based on the results of a previous genome-wide association study. Per-allele SNP associations were assessed by a Cox regression to model the cumulative dose of nab-PTX up to the onset of severe or worsening sensory neuropathy. A total of 141 patients were enrolled in the parent study; 91(65%) were included in this sub-study. Worsening of CIPN was significantly greater in the cases with *XKR4* AC compared to those with a homozygote AA (HR 1.86, 95%CI: 1.00001−3.46, $p = 0.049$). There was no significant correlation of CIPN with any other SNP. A multivariate analysis showed that the cumulative dose of nab-PTX was most strongly correlated with CIPN ($p < 0.01$).

Taxane-based regimens for metastatic breast cancer (MBC) are standard therapies that have demonstrated clinical benefit, but chemotherapy-induced peripheral neuropathy (CIPN) is a major clinical challenge due to the lack of an effective treatment for it, plus the negative impact that CIPN exerts on patients' quality of life (QOL) [1-3]. Taxanes inhibit the depolymerization of microtubules, and the resulting abnormal aggregation of microtubules in neuronal cells may cause CIPN [4,5]. Polyoxyethylated castor oil, which is used as a vehicle for the taxane paclitaxel (PTX), may also increase the risk for prolonged peripheral neuropathy [6].

Nab-paclitaxel (nab-PTX) is an albumin-bound, 130-nm particle formulation of PTX [7]. Formulation with albumin allows nab-PTX to be reconstituted as a simple saline solution without the need for another solvent. Thus, at the time of the development of nab-PTX, the incidence of CIPN with nab-PTX was expected to be lower than that with conventional, solvent-based PTX (so-PTX) [8]. However, in a phase III study comparing 3-week cycles (q3w) of nab-PTX (260 mg/m^2) with q3w so-PTX (175 mg/m^2) in patients with MBC, CIPN occurred more frequently in the nab-PTX arm [9]. Another phase III study also showed that grade ≥ 3 CIPN was more frequent in the weekly nab-PTX arm compared to the weekly so-PTX arm, with dose reductions and discontinuation due to CIPN being more common and occurring earlier for patients treated with nab-PTX [10].

In Japan, nab-PTX was approved in 2010 for the treatment of MBC. The dosage of nab-PTX recommended by Japan's Pharmaceuticals and Medical Devices Agency is 260 mg/m^2 administered intravenously over a 30-min period every 3 weeks. Data obtained in post-marketing-surveillance showed that one-third of patients received a reduced dose of nab-PTX, even in their first course of treatment, and almost 30% of the patients treated with the recommended dose were also dose-reduced in a later course; the main causes of dose reduction were myelosuppression and CIPN [11]. CIPN occurred frequently (grade ≥ 2: 42.5%, grade ≥ 3: 10.8%) and was shown to be a serious problem affecting the clinical utility and efficacy of nab-PTX. Although it is clear that CIPN impairs patients' QOL and may reduce the benefits of treatment, the clinical symptoms and severity of CIPN differ among patients, and this suggests that individual characteristics might affect the susceptibility to CIPN. However, it is not yet possible to predict a patient's risk for developing CIPN. Clarifying this risk is this important for both patients and physicians, and doing so may contribute to preventive and therapeutic interventions for CIPN.

Some risk factors for the occurrence and severity of CIPN have been identified: the cumulative doses of taxanes, patient age, obesity, and ethnic differences. A genome-wide association study (GWAS) identified several genetic markers of PTX-induced peripheral neuropathy and suggested that the single nucleotide polymorphisms (SNPs) *EPHA4* (rs17348202), *EPHA5* (rs7349683), *LIMK 2* (rs5749248), and *XKR4* (rs4737264) are associated with the onset or severity of peripheral neuropathy [12-14]. The association of PTX-induced peripheral neuropathy with these SNPs in Japanese patients has not been examined. The ABROAD trial was performed as a phase II study of q3w nab-PTX in an effort to identify the optimal dose of nab-PTX in patients in Japan with MBC [15]. In the present substudy of the ABROAD trial, we investigated genetic risk factors for the development and severity of nab-PTX-induced sensory neuropathy, with the goal of identifying the relationships between this condition and SNPs in Japanese patients.

Patients and Methods

Study design.   The ABROAD trial was a multicenter, open-label, randomized phase II trial for patients with human epidermal growth factor receptor 2 (HER2)-negative MBC; the details of the trial's design were reported [15]. Briefly, the key inclusion criteria for the trial were pathologically confirmed invasive carcinoma; incurable metastatic disease; age 20-75 years; an Eastern Cooperative Oncology Group performance status of 0 or 1; and chemotherapy for MBC (up to one regimen). The key exclusion criteria were HER2-positive breast cancer; patients with grade ≥ 2 sensory

neuropathy; and brain metastases with symptoms or requiring treatment.

Eligible patients were randomly assigned in a 1 : 1 : 1 ratio in the ABROAD trial to receive any dose of nab-PTX (SD (standard dose): 260 mg/m^2, MD (medium dose): 220 mg/m^2, LD (low dose): 180 mg/m^2) once every 3 weeks for 30 min as one course. The treatment was continued until disease progression or an unbearable adverse event occurred. All patients provided their written informed consent before enrollment in the present sub-study, which was approved by the institutional ethics committee on human research at all participating medical centers and was conducted in accord with the Declaration of Helsinki and Japan's ethical guidelines for epidemiology research and human genome/gene analysis research.

Assessment of CIPN.   The evaluation of peripheral neuropathy was performed at each patient's trial registration (baseline) and during the second, fourth, and sixth courses of the protocol treatment with nab-PTX. A physician-based assessment of CIPN was performed using the sensory neuropathy subscales of the U.S. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. These subscales were used by the physicians to report the severity of a patient's neuropathy based on four grades, with a higher number indicating increased severity: Grade 1 (no symptoms and only clinical or laboratory findings), Grade 2 (moderate), Grade 3 (advanced), and Grade 4 (life-threatening).

Patient-reported outcomes (PROs) related to CIPN were surveyed using the Patient Neurotoxicity Questionnaire (PNQ) at each time point [16]. The PNQ is a self-administered questionnaire comprising two items on sensory and motor neurotoxicity. The PNQ grades range from A (no neuropathy) to E (very severe neuropathy). There is a clear demarcation between PNQ grades A to C and grades D to E, which indicate CIPN symptoms that do not and do interfere with activities of daily living, respectively. The original PNQ was developed in English, and a Japanese version has subsequently been validated [17].

Genotyping.   A 1-mL blood sample was collected from each patient at enrollment in the trial. DNA was extracted and the concentration was fixed at 10 ng/µL. The DNA samples were stored at −80°C. Genotyping was performed for eligible patients who had sufficient DNA in the sample, with the use of an ABI PRISM 7700 system and the protocol in the TaqMan® Sample-to-SNP™ Kit (Applied Biosystems, Carlsbad, CA, USA). The SNPs analyzed were *EPHA4*-rs17348202, *EPHA5*-rs7349683, *EPHA6*-rs301927, *LIMK2*-rs5749248, and *XKR4*-rs4737264 based on an earlier GWAS [14]. The genotyping was performed twice at a single facility (Okayama University Hospital) for quality control. The neuropathy data were managed at the Comprehensive Support Project for Oncological Research data center and were not disclosed to those performing the measurements.

Statistics.   The parent study/ABROAD trial was planned to ensure the selection of an MD with a probability of 70%, based on the 1-year progression-free survival (PFS) of 30% of the cases at all three doses and grade 3 neurotoxicity rates of the SD, MD, and LD groups at 15%, 8%, and 0.1%, respectively [15]. This required 40 patients per group with expected registration periods of 2 years and follow-up periods of 2 years. We eventually included 42 patients per group. Since the present study is an exploratory sub-analysis of the parent study, the sample size was not designed in the sub-study, and the sub-study was conducted with the policy of recruiting as many subjects from the parent trial as possible.

The associations between the five SNPs and CIPN were examined by a survival analysis using the cumulative nab-PTX dose (dose per body or dose per body surface area) until the occurrence of CIPN-related events as an outcome. These events were defined as follows: (1) grade ≥2 CIPN diagnosed based on CTCAE v.4.0 by a physician, (2) a worsening CIPN grade based on CTCAE v.4.0, and (3) a PNQ sensory grade of D or E. Cases without a CIPN-related event at the last observation point were treated as censored on the time axis at the cumulative dose.

Cumulative dose-to-event data were investigated with the use of Kaplan-Meier curves, and a log-rank test was used for the comparisons between alleles. The hazard ratio (HR) and 95% confidence interval (95%CI) per allele were calculated by univariate and multivariate Cox regression analyses. The explanatory variables for multivariate Cox regression included the five genes. The relationship of each SNP with the CIPN grade based on CTCAE v.4.0 was examined by a multivariate multinominal logistic regression analysis with a proportional odds model, and an odds ratio (OR) and 95%CI were calculated. In this model, the cumulative dose of nab-

PTX was added as an explanatory variable, along with the SNPs. SAS ver. 9.3 software was used for all analyses. Probability (*p*)-values < 0.05 were considered significant.

Clinical trial registration.   The protocol was registered at the website of the University Hospital Medical Information Network (UMIN), Japan (protocol ID: UMIN000015516), on November 1, 2014. Details are available at the following address: https: //upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno= R000017916.

Results

Patient characteristics.   A total of 141 Japanese patients were enrolled in the ABROAD trial from February 2015 to June 2018 and were randomly assigned to the SD (260 mg/m^2), MD (220 mg/m^2), and LD (180 mg/m^2) groups. Of the 141 patients, 91 (65% of the entire study population) agreed to participate in the present sub-study (Fig. 1). The characteristics of these 91 patients are summarized in Table 1. There were 29 patients in the SD group, 28 in the MD group, and 34 in the LD group. The median age was 60 (range 34-74) years, and the median body mass index (BMI) was 23.2 (15-37) kg/m^2. Among the 91 cases, 16 (17%) had dose reductions because of an adverse effect (AE), and 22 patients (24%) discontinued treatment due to an AE (including the patient's decision). A breakdown of the AEs that caused dose reduction or treatment dis-continuation was not recorded.

Allele frequencies.   The genotype distributions of rs17348202, rs7349683, rs301927, rs5749248, and rs4737264 are shown in Table 2. Four of the distributions were in Hardy-Weinberg equilibrium, but for rs17348202 the genotypes of all of the patients were homozygous for the major allele, and thus statistical analyses were not conducted. The minor allele frequencies (MAFs) were 0.43 for rs7349683, 0.36 for rs301927, 0.07 for rs5749248, and 0.10 for rs4737264. The patient background for each gene is also shown in Table 2. The only significant differences were for age and BMI for the *LIMK2*-rs5749248 genotype: the patients with a homozygote A/A were significantly older (61 vs. 56 years, *p* = 0.04) and had a significant higher BMI (23.5 vs. 20.6 kg/m^2, *p* < 0.01) compared to the patients with a heterozygote A/C.

Associations between SNPs and CIPN-related events.   The *EPHA4*-rs17348202 genotype was homozygous for the major allele in all patients and was therefore excluded from the analysis. As pre-defined CIPN-related events, grade ≥ 2 CIPN on CTCAE v.4.0 was observed in 45 cases (49%), a worsening CIPN grade on CTCAE v.4.0 was observed in 76 cases (84%), and a PNQ sensory grade of D or E was observed in 29 cases (32%). Figure 2 provides the Kaplan-Meier curves for the cumulative dose relative to these CIPN-related events (grade ≥ 2 CIPN, a worsening CIPN grade, or a PNQ sensory grade of D or E) for each genotype. A log rank-test showed no significant differences for geno-
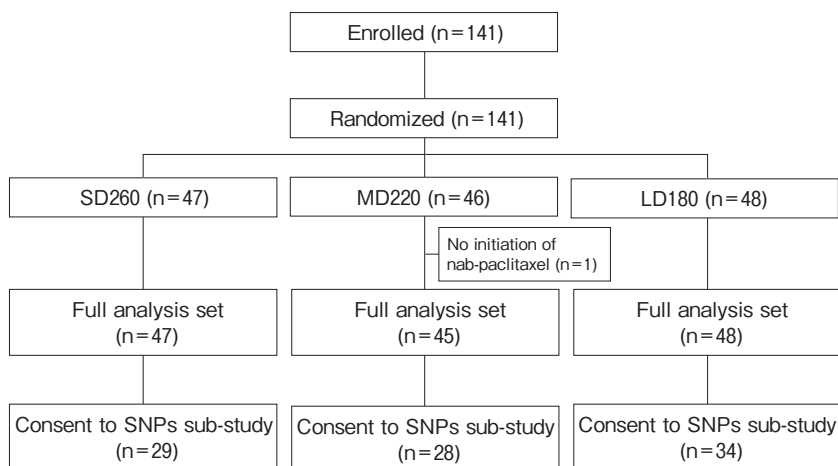

Fig. 1   Consort diagram. SD 260 (260 mg/m^2), MD 220 (220 mg/m^2), and LD 180 (180 mg/m^2) of 3-week-cycle nab-paclitaxel. SNP, single nucleotide polymorphism.

Table 1   Patient characteristics

Item	All patients (n = 91)		SD 260 (n = 29)		MD 220 (n = 28)		LD 180 (n = 34)	
	Number of patients	(%)	Number of patients	(%)	Number of patients	(%)	Number of patients	(%)
Treatment								
SD 260	29	(32)						
MD 220	28	(31)						
LD 180	34	(37)						
Age (years)								
Median (range)	60.0	(34–74)	58.0	(41–73)	65.0	(34–72)	59.0	(35–74)
BMI (kg/m^2)								
Median (range)	23.2	(15–37)	25.3	(18–37)	23.2	(18–37)	21.8	(15–29)
Baseline ECOG performance status								
0	69	(76)	22	(76)	22	(79)	25	(73.5)
1	21	(23)	7	(24)	5	(18)	9	(27)
Missing	1	(1)	0	(0)	1	(3.6)	0	(0)
Estrogen receptor								
Positive	69	(76)	21	(72)	21	(75)	27	(79)
Negative	20	(22)	8	(27)	6	(21)	6	(18)
Missing	2	(2)	0	(0)	1	(4)	1	(3)
Pre-treatment by taxane								
No	50	(55)	4	(14)	9	(32)	10	(29)
Yes	41	(45)	25	(86)	19	(68)	24	(71)

SD 260, 260 mg/m^2 of 3-week cycle nab-paclitaxel; MD 220, 220 mg/m^2 of 3-week cycle nab-paclitaxel; LD 180, 180 mg/m^2 of 3-week cycle nab-paclitaxel; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group.

Table 2   Distribution of genotypes

	EPHA5–rs7349683 (n = 91)				EPHA6–rs301927 (n = 91)				LIMK2–rs5749248 (n = 91)				XKR4–rs4737264 (n = 91)			
Genotype	TT (n=28, 31%)	TC (n=47, 52%)	CC (n=16, 18%)	P value	AA (n=15, 16%)	AG (n=37, 41%)	GG (n=39, 43%)	P value	AA (n=79, 87%)	AC (n=12, 13%)	CC (n=0)	P value	AA (n=72, 79%)	AC (n=19, 21%)	CC (n=0)	P value
Treatment, n (%)				0.92[†]				0.98[†]				0.95[†]				0.20[†]
SD 260	10 (36)	13 (28)	6 (38)		5 (33)	12 (32)	12 (30)		25 (31)	4 (33)	–		23 (32)	6 (31)	–	
MD 220	8 (28)	15 (32)	5 (31)		5 (33)	12 (32)	11 (28)		24 (30)	4 (33)	–		25 (35)	3 (16)	–	
LD 180	10 (36)	19 (40)	5 (31)		5 (33)	13 (35)	16 (41)		30 (38)	4 (33)	–		24 (33)	10 (53)	–	
Age (years)				0.94[*]				0.32[*]				0.04[*]				0.43[*]
Median (range)	59.5 (35–73)	59.0 (34–74)	60.5 (40–72)		64.0 (41–74)	61.0 (35–74)	59.0 (34–72)		61.0 (35–74)	56.5 (34–66)	–		59.0 (34–74)	61.0 (39–74)	–	
BMI (kg/m^2)				0.06[*]				0.31[*]				<0.01[*]				0.82[*]
Median (range)	23.3 (17–36)	23.5 (15–37)	21.8 (18–26)		24.0 (18–37)	23.5 (18–32)	21.8 (15–37)		23.5 (17–37)	20.6 (15–24)	–		22.9 (15–37)	23.5 (18–36)	–	
Pre-treatment by taxane, n (%)				0.19[†]				0.49[†]				0.38[†]				0.46[†]
Yes	16 (57)	17 (36)	8 (50)		8 (53)	19 (51)	25 (64)		37 (47)	4 (33)	–		31 (43)	10 (53)	–	
No	12 (43)	30 (64)	8 (50)		7 (27)	18 (49)	14 (36)		42 (53)	8 (67)	–		41 (57)	9 (47)	–	
ECOG PS, n (%)				0.03[†]				0.07[†]				0.63[†]				0.59[†]
0	24 (86)	37 (79)	8 (50)		8 (54)	29 (78)	32 (82)		61 (77)	8 (67)	–		53 (74)	3 (16)	–	
1	4 (14)	10 (21)	7 (44)		6 (40)	8 (21)	7 (18)		17 (21)	4 (33)	–		18 (25)	16 (84)	–	
Missing	–	–	1 (6)		1 (6)	–	–		1 (2)	–	–		1 (1)	–	–	

NOTE. [†]Chi-square test. [*]Kruskal-Wallis test. *EPHA4*-rs17348202 was excluded because no genetic polymorphism was found.
SD 260, 260 mg/m^2 of 3-week cycle nab-paclitaxel; MD 220, 220 mg/m^2 of 3-week cycle nab-paclitaxel; LD 180, 180 mg/m^2 of 3-week cycle nab-paclitaxel; BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group perf.

types rs7349683, rs301927, rs5749248, and rs4737264 in cumulative nab-PTX dose (mg per body surface area) relative to the rate of CIPN-related event-free survival or in the cumulative nab-PTX dose itself (data not shown).

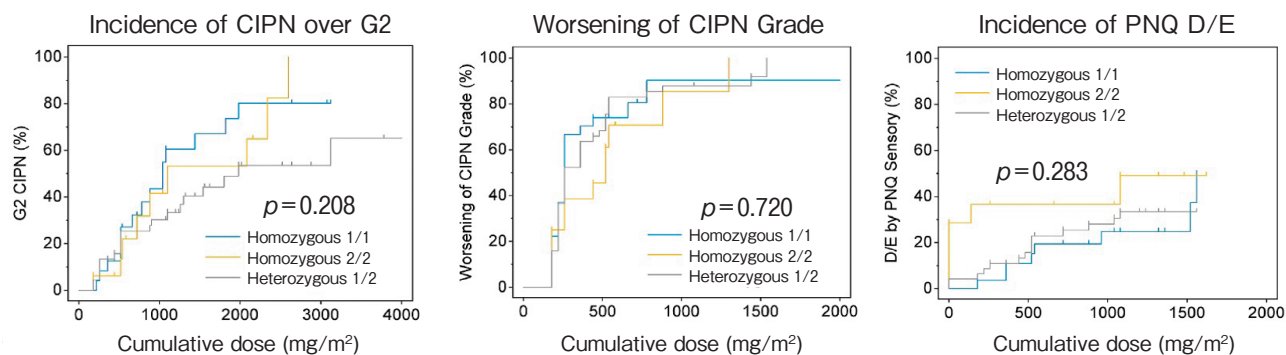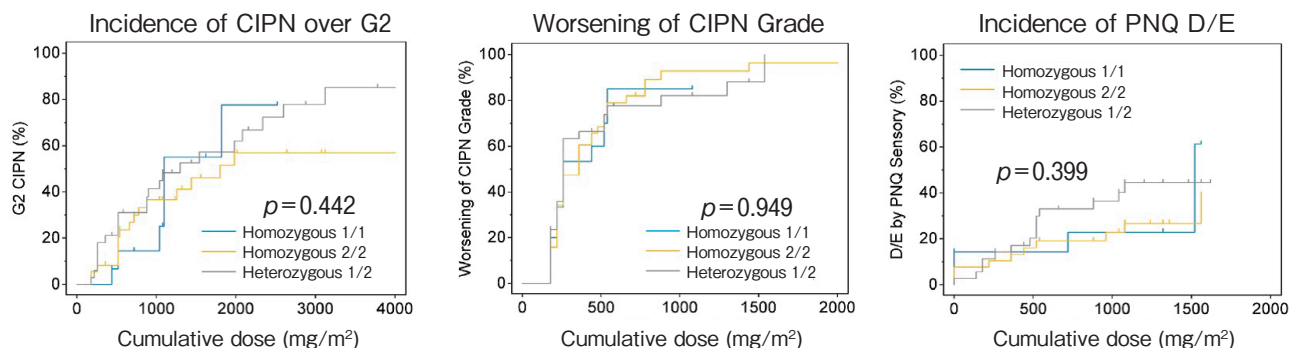Table 3 shows the results of the multivariate Cox

A. *EPHA5*-rs7349683

B. *EPHA6*-rs301927

Fig. 2   Kaplan-Meier curves for the cumulative dose to chemotherapy-induced peripheral neuropathy (CIPN)-related events in genotypes (A) *EPHA5*-rs7349683, (B) *EPHA6*-rs301927, (C) *LIMK2*-rs5749248, and (D) *XKR4*-rs4737264. CIPN-related events were defined as grade ≥2 CIPN diagnosed based on CTCAE v.4.0 by a physician, a worsening CIPN grade based on CTCAE v.4.0, and a Patient Neurotoxicity Questionnaire sensory grade of D or E. Y-axis: the cumulative incidence of CIPN-related events. X-axis: the cumulative dose (mg per body surface area) of nab-paclitaxel. *P*-values are from log rank tests.

regression analysis of the relationship between the cumulative nab-PTX dose (mg/m²) and CIPN-related event-free survival; these results demonstrate that there were no significant associations between the five SNPs and CIPN-related events. The multivariate Cox regression for the cumulative nab-PTX dose (mg/body) showed a significant difference in the worsening of CIPN for the *XKR4*-rs4737264 genotype (HR for heterozygous A/C: 1.86, 95%CI: 1.00001-3.46, *p* = 0.049) (Table 4). This result indicates that the genetic polymorphism of *XKR4*-rs4737264 may be associated with worsening CIPN.

To test this relationship, we performed an additional analysis with the assigned treatment group as an explanatory variable for the Cox model (Table 5). This

model for the cumulative nab-PTX dose (mg/body) showed that the SNP *XKR4*-rs4737264 was also significantly associated with worsening of CIPN (HR for heterozygous A/C: 1.90, 95%CI: 1.01-3.57, *p* = 0.047). A similar tendency was observed in the analysis of the relationship between the accumulated dose per body surface area and CIPN (data not shown).

Associations between the SNPs and the severity of CIPN.   As mentioned above, the *EPHA4*-rs17348202 genotype was homozygous for the major allele in all patients and was thus excluded from the analysis. The multivariate multinomial logistic regression revealed no significant association between any of the SNPs and the grade of CIPN (Table 6). The cumulative dose of nab-PTX was the only factor significantly associated with the

C. *LIMK2*-rs5749248
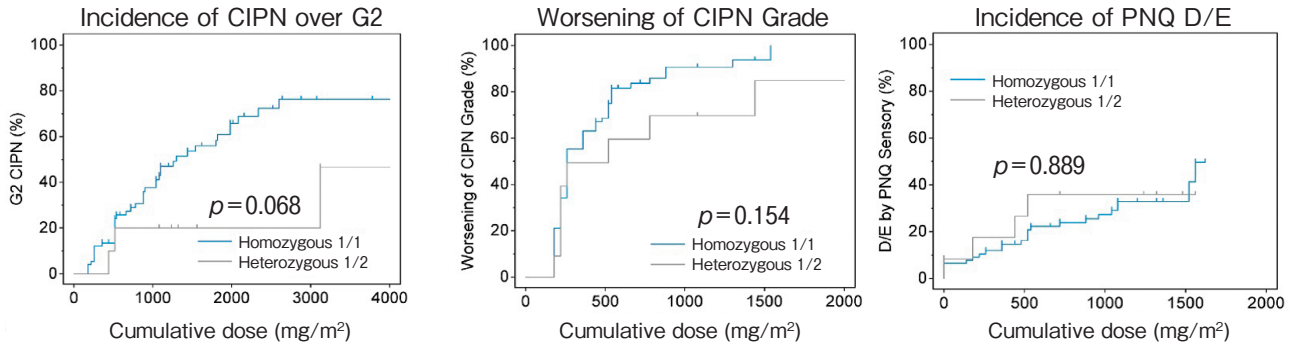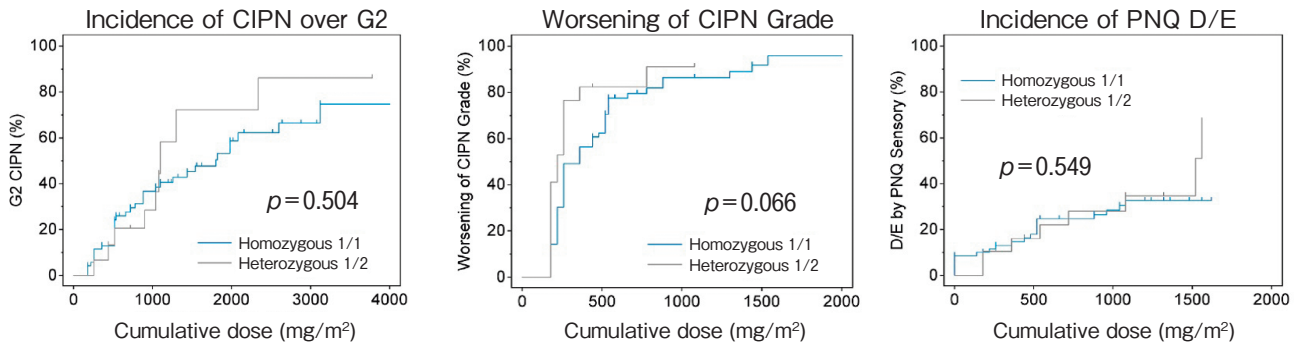

D. *XKR4*-rs4737264

Fig. 2   Kaplan-Meier curves for the cumulative dose to chemotherapy-induced peripheral neuropathy (CIPN)-related events in genotypes (A) *EPHA5*-rs7349683, (B) *EPHA6*-rs301927, (C) *LIMK2*-rs5749248, and (D) *XKR4*-rs4737264. CIPN-related events were defined as grade ≥2 CIPN diagnosed based on CTCAE v.4.0 by a physician, a worsening CIPN grade based on CTCAE v.4.0, and a Patient Neurotoxicity Questionnaire sensory grade of D or E. Y-axis: the cumulative incidence of CIPN-related events. X-axis: the cumulative dose (mg per body surface area) of nab-paclitaxel. *P*-values are from log rank tests.

severity of CIPN (multivariate OR: 2.33, 95%CI: 1.62-3.34, $p < 0.0001$).

Discussion

We examined the relationship between the onset and severity of CIPN and five SNPs that have been associated with CIPN [14]. *EPHA4* had no genetic polymorphism in the present patients and was excluded from the analysis. The *XKR4* genotype was observed to be significantly associated with CIPN, with a significantly worse CIPN grade than that at baseline in the patients with the *XKR4*-rs4737264 heterozygous allele AC compared to those with AA. No association was revealed between the *XKR4* genotype and other CIPN-related

events (grade ≥2 CIPN on CTCAE v.4.0 or a PNQ sensory grade of D or E). We speculate that this result was influenced by the detection sensitivity due to the difference in the number of events. There was no significant association between the onset or severity of CIPN with genetic polymorphisms in *EPHA5*-rs7349683, *EPHA6*-rs301927, and *LIMK2*-rs5749248.

CIPN is a serious adverse event that may interfere with drug efficacy, and the identification of risk factors for CIPN's onset and severity is important for patients and physicians, and for establishing preventive and therapeutic approaches. Older age, obesity, and ethnic differences are risk factors for CIPN, and African patients may have a higher incidence of CIPN [13]; there have been few studies of CIPN in Japanese

Table 3 Cox regression for a cumulative dose (mg/m²) relative to CIPN-related event free survival

| | Incidence of CIPN Grade ≥2 (CTCAE v.4.0) | | | | | | | Worsening of CIPN Grade (CTCAE v.4.0) | | | | | | | Incidence of PNQ Grade D/E | | | | | | |
| | Univariate | | | Multivariate* | | | | Univariate | | | Multivariate* | | | | Univariate | | | Multivariate* | | | |
	Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value		Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value		Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value	
EPHA5-rs7349683																					
Homozygous T/T	ref			ref				ref			ref				ref			ref			
Heterozygous T/C	0.57	(0.29, 1.10)	0.098	0.58	(0.29, 1.15)	0.116		0.94	(0.56, 1.58)	0.813	1.02	(0.58, 1.79)	0.935		1.15	(0.48, 2.77)	0.749	1.10	(0.45, 2.70)	0.832	
Homozygous C/C	0.88	(0.38, 2.02)	0.767	0.73	(0.30, 1.76)	0.483		0.74	(0.37, 1.51)	0.409	0.89	(0.40, 2.00)	0.784		2.26	(0.78, 6.54)	0.133	2.06	(0.67, 6.35)	0.210	
EPHA6-rs301927																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/G	1.37	(0.55, 3.41)	0.501	1.42	(0.57, 3.56)	0.456		1.08	(0.54, 2.19)	0.821	1.05	(0.51, 2.14)	0.901		1.50	(0.49, 4.58)	0.474	1.43	(0.47, 4.41)	0.530	
Homozygous G/G	0.91	(0.36, 2.35)	0.851	1.02	(0.38, 2.74)	0.970		1.10	(0.56, 2.17)	0.787	1.20	(0.58, 2.46)	0.623		0.88	(0.27, 2.81)	0.827	1.00	(0.30, 3.38)	1.000	
LIMK2-rs5749248																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/C	0.35	(0.11, 1.15)	0.083	0.40	(0.12, 1.37)	0.145		0.59	(0.28, 1.25)	0.168	0.52	(0.24, 1.13)	0.100		1.08	(0.37, 3.13)	0.882	1.10	(0.37, 3.25)	0.861	
Homozygous C/C	-			-				-			-				-			-			
XKR4-rs4737264																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/C	1.27	(0.61, 2.66)	0.521	1.08	(0.49, 2.40)	0.842		1.74	(0.98, 3.09)	0.058	1.85	(1.00, 3.44)	0.050		1.27	(0.56, 2.90)	0.567	1.29	(0.53, 3.12)	0.574	
Homozygous C/C	-			-				-			-				-			-			

NOTE. * In multivariate Cox regression, four single nucleotide polymorphisms were added to the model as explanatory variables.
CIPN, chemotherapy-induced peripheral neuropathy; PNQ, Patient Neurotoxicity Questionnaire; CTCAE v.4.0, Common Terminology Criteria for Adverse Events version 4.0; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 4 Cox regression for cumulative dose (mg/body) relative to CIPN-related event free survival

| | Incidence of CIPN ≥ Grade 2 (CTCAE v.4.0) | | | | | | | Worsening of CIPN Grade (CTCAE v.4.0) | | | | | | | Incidence of PNQ Grade D/E | | | | | | |
| | Univariate | | | Multivariate* | | | | Univariate | | | Multivariate* | | | | Univariate | | | Multivariate* | | | |
	Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value		Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value		Estimate	HR 95% CI	P-value	Estimate	HR 95% CI	P-value	
EPHA5-rs7349683																					
Homozygous T/T	ref			ref				ref			ref				ref			ref			
Heterozygous T/C	0.56	(0.29, 1.10)	0.093	0.57	(0.29, 1.14)	0.111		0.90	(0.53, 1.52)	0.699	0.99	(0.57, 1.74)	0.978		1.11	(0.47, 2.65)	0.812	1.08	(0.44, 2.62)	0.871	
Homozygous C/C	0.99	(0.43, 2.26)	0.973	0.83	(0.34, 2.00)	0.672		0.79	(0.39, 1.61)	0.519	0.98	(0.43, 2.20)	0.955		2.31	(0.79, 6.71)	0.124	2.11	(0.68, 6.56)	0.195	
EPHA6-rs301927																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/G	1.43	(0.58, 3.54)	0.443	1.45	(0.58, 3.63)	0.431		1.11	(0.55, 2.25)	0.762	1.11	(0.55, 2.26)	0.772		1.69	(0.54, 5.31)	0.368	1.65	(0.52, 5.28)	0.398	
Homozygous G/G	0.95	(0.37, 2.44)	0.919	1.09	(0.41, 2.93)	0.864		1.13	(0.57, 2.23)	0.722	1.28	(0.62, 2.64)	0.509		0.98	(0.30, 3.16)	0.969	1.14	(0.33, 3.97)	0.837	
LIMK2-rs5749248																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/C	0.36	(0.11, 1.20)	0.096	0.42	(0.12, 1.44)	0.167		0.61	(0.29, 1.29)	0.198	0.55	(0.25, 1.17)	0.121		1.19	(0.41, 3.44)	0.752	1.17	(0.40, 3.46)	0.775	
Homozygous C/C	-			-				-			-				-			-			
XKR4-rs4737264																					
Homozygous A/A	ref			ref				ref			ref				ref			ref			
Heterozygous A/C	1.30	(0.62, 2.73)	0.485	1.15	(0.52, 2.55)	0.728		1.72	(0.97, 3.05)	0.065	1.86	(1.00001, 3.46)	0.049		1.24	(0.54, 2.85)	0.605	1.31	(0.53, 3.24)	0.554	
Homozygous C/C	-			-				-			-				-			-			

NOTE. * In multivariate Cox regression, four single nucleotide polymorphisms were added to the model as explanatory variables.
CIPN, chemotherapy-induced peripheral neuropathy; PNQ, Patient Neurotoxicity Questionnaire; CTCAE v. 4.0, Common Terminology Criteria for Adverse Events version 4.0; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 5　Cox regression for a cumulative dose relative to worsening of CIPN event free survival with assigned treatment groups as explanatory variables

	Worsening of CIPN Grade (CTCAE v. 4.0)											
	model 1[#]						model 2[#]					
	Univariate			Multivariate*			Univariate			Multivariate*		
	HR			HR			HR			HR		
	Estimate	95%CI	P-value	Estimate	95%CI	P-value	Estimate	95%CI	P-value	Estimate	95%CI	P-value
Treatment group												
LD 180	ref			ref			ref			ref		
MD 220	0.90	(0.51, 1.61)	0.732	0.90	(0.49, 1.64)	0.736	0.89	(0.50, 1.58)	0.685	0.87	(0.48, 1.59)	0.650
SD 260	0.88	(0.51, 1.52)	0.652	0.89	(0.50, 1.59)	0.702	0.8	(0.48, 1.42)	0.491	0.81	(0.46, 1.43)	0.467
EPHA5-rs7349683												
Homozygous T/T	ref			ref			ref			ref		
Heterozygous T/C	0.94	(0.56, 1.58)	0.813	1.00	(0.56, 1.77)	0.989	0.90	(0.53, 1.52)	0.699	0.95	(0.53, 1.68)	0.854
Homozygous C/C	0.74	(0.37, 1.51)	0.409	0.90	(0.40, 2.01)	0.800	0.79	(0.39, 1.61)	0.519	1.00	(0.44, 2.25)	0.998
EPHA6-rs301927												
Homozygous A/A	ref			ref			ref			ref		
Heterozygous A/G	1.08	(0.54, 2.19)	0.821	1.05	(0.52, 2.16)	0.883	1.11	(0.55, 2.25)	0.762	1.12	(0.55, 2.29)	0.750
Homozygous G/G	1.10	(0.56, 2.17)	0.787	1.20	(0.58, 2.46)	0.623	1.13	(0.57, 2.23)	0.722	1.29	(0.63, 2.67)	0.489
LIMK2-rs5749248												
Homozygous A/A	ref			ref			ref			ref		
Heterozygous A/C	0.59	(0.28, 1.25)	0.168	0.52	(0.24, 1.14)	0.101	0.61	(0.29, 1.29)	0.198	0.54	(0.25, 1.18)	0.126
Homozygous C/C	–			–			–			–		
XKR4-rs4737264												
Homozygous A/A	ref			ref			ref			ref		
Heterozygous A/C	1.74	(0.98, 3.09)	0.058	1.87	(1.00, 3.50)	0.052	1.72	(0.97, 3.05)	0.065	1.90	(1.01, 3.57)	0.047
Homozygous C/C	–			–			–			–		

NOTE. [#]Cox regression for cumulative dose of nab-paclitaxel (model 1: mg/m^2, model 2: mg/body) relative to worsening if CIPN event free.

*In multivariate Cox regression, four single nucleotide polymorphisms and assigned treatment groups were added to the model as explanatory variables.

CIPN, chemotherapy-induced peripheral neuropathy; CTCAE v. 4.0, Common Terminology Criteria for Adverse Events version 4.0; HR, hazard ratio; 95%CI, 95% confidential interval; LD 180, 180 mg/m^2 of 3-week cycle nab-paclitaxel; MD 220, 220 mg/m^2 of 3-week cycle nab-paclitaxel; SD 260, 260 mg/m^2 of 3-week cycle nab-paclitaxel.

Table 6　Multivariate multinomial logistic regression for CIPN grade

| | Univariate | | | Multivariate* | | |
| | OR | | | OR | | |
	Estimate	95% CI	P-value	Estimate	95% CI	P-value
EPHA5-rs7349683						
Homozygous T/T	ref			ref		
Heterozygous T/C	0.75	(0.32, 1.77)	0.513	1.29	(0.51, 3.28)	0.589
Homozygous C/C	0.99	(0.31, 3.15)	0.982	1.11	(0.32, 3.85)	0.875
EPHA6-rs301927						
Homozygous A/A	ref			ref		
Heterozygous A/G	1.91	(0.60, 6.11)	0.276	2.95	(0.86, 10.09)	0.085
Homozygous G/G	1.13	(0.36, 3.53)	0.832	1.82	(0.51, 6.41)	0.353
LIMK2-rs5749248						
Homozygous A/A	ref			ref		
Heterozygous A/C	0.46	(0.15, 1.44)	0.181	0.48	(0.13, 1.80)	0.277
Homozygous C/C	–			–		
XKR4-rs4737264						
Homozygous A/A	ref			ref		
Heterozygous A/C	1.33	(0.52, 3.40)	0.555	1.70	(0.56, 5.17)	0.347
Homozygous C/C	–			–		
Log$_2$ (cumulative dose mg/m^2)	2.22	(1.56, 3.16)	<0.0001	2.33	(1.62, 3.34)	<0.0001

NOTE. Chemotherapy-induced peripheral neuropathy was classified into Grades 1 to 4 based on the Common.

Terminology Criteria for Adverse Events. *In multivariate multinominal logistic regression, four single nucleotides polymorphisms and cumulative dose of nab-paclitaxel (mg/m^2) were added to the model as explanatory variables.

CIPN, chemotherapy-induced peripheral neuropathy; OR, odds ratio; 95% CI, 95% confidence interval.

patients. Tanabe *et al.* reported that an SNP in ABCB1 and older age might be predictors of PTX-induced peripheral neuropathy [18], but it is unclear whether these findings apply to nab-PTX. To the best of our knowledge, the present study is the first to examine the relationship between SNPs in Japanese patients and the development of CIPN after treatment with nab-PTX.

The genes examined in our present analysis, *i.e.*, *EPHA4*-rs17348202, *EPHA5*-rs7349683, *EPHA6*-rs301927, *LIMK2*-rs5749248, and *XKR4*-rs4737264, were described as associated with CIPN in an earlier study [14]. Eph receptors produced by *EPHA4/5/6* are important in the development of the nervous system and the repair of nerve cells. *EPHA4* is related to developmental processes and astrocyte responsiveness in the nervous system, and it may determine nervous system vulnerability due to axonal degeneration [19]. *EPHA5* is involved in the early stages of synaptogenesis [20], and *EPHA6* has been linked to memory and learning ability [21]. *LIMK2* affects the development of the nervous system by controlling actin dynamics. *LIMK2* knockout mice exhibit minimal abnormalities, but *LIMK*-1/2 double-knockout mice have impaired excitatory synaptic function [22]. Knockdown of *LIMK2* results in significant reductions of the neurite bearing cell count, neurite length, and cone extension growth rate [23,24]. *XKR4* belongs to the XKR family, and rs4737264, an intronic SNP in *XKR4*, has been associated with hyperactivity disorder, but the details are unknown [25].

The five genes examined herein have been reported as risk factors for CIPN in Western countries, but our present investigation identified only the SNP in *XKR4* as a risk factor for CIPN. This discrepancy may be due to the small number of samples, ethnic differences, and few SNP variations. The frequency of SNP expression in the present study also differs from those in evaluations performed in Europe and the USA. The MAFs in the 1000 Genomes Project and in our study were 0.043 and 0 for *EPHA4*-rs7349683, 0.67 and 0.43 for *EPHA5*-rs7349683, 0.55 and 0.36 for *EPHA6*-rs301927, 0.12 and 0.66 for *LIMK2*-rs5749248, and 0.15 and 0.10 for *XKR4*-rs4737264 [26]. The frequency of SNPs varies depending on ethnic differences, and this difference may affect the frequency of CIPN onset by ethnic differences [27].

Another important finding of our sub-analysis of the ABROAD trial is that the cumulative dose of nab-PTX,

rather than SNPs, was the strongest risk factor for CIPN severity. The ABROAD trial also showed that the lower dose of 180 mg/m^2 was better than the current standard dose for metastatic breast cancer in Japanese patients, based on the PFS and the incidence of CIPN [28]. Therefore, the dose of nab-PTX should be carefully adjusted to reduce the risk of the onset of CIPN and maintain patients' QOL.

There are several limitations in this study, including the small number of cases (n=91), the limited multivariate analysis, and the non-exhaustive analysis of only five genes. There may be other genetic mutations that are more relevant. Within these limitations, our results revealed that the *XKR4*-rs4737264 genotype is associated with nab-PTX-induced peripheral neuropathy in Japanese patients with metastatic breast cancer. However, the main risk factor for CIPN is the cumulative dose of nab-PTX. A study of the optimal dosing of nab-PTX for good clinical efficacy and tolerability in Japanese patients is thus warranted to mitigate the risk of CIPN.

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