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Efficacy and safety of a modified combination regimen of phenothrin and ivermectin lotion in patients with head lice in Tsukuba, Japan

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Abstract

Objectives: The combination therapy of phenothrin (PHT) and ivermectin (PI regimen) was effective for pyrethroid-resistant head lice in Okinawa; however, further investigations have been required to improve the regimen. This study aimed to investigate the efficacy of the modified PI (mPI) regimen, which increased the maximum number of PHT treatments in the PI regimen by one, in Tsukuba, which has a low mutation rate compared to Okinawa.

Methods: An open-label, uncontrolled, exploratory study was conducted at the Department of Dermatology, University of Tsukuba Hospital to investigate the treatment effect of mPI regimen. Genotypes were investigated for the knockdown (*kdr*) mutations at voltage-sensitive sodium channel (VSSC) and glutamate-gated chloride receptor-encoding loci to identify their correlation with drug resistance.

Results: All the 8 patients treated with the mPI regimen resulted in lice free on day 29. Compared to baseline, the number of bodies, eggs, and itching grade significantly decreased on day 29. Mild erythema appeared after the second application of PHT on day 8 in one patient, persisted for 2 days, and recovered on day 15. No other adverse events were reported. Mutation rates were more than double in head lice collected from one patient for whom PHT was ineffective compared to those in lice from patients who responded to PHT. Furthermore, we identified four new haplotypes of VSSC loci in head lice.

Conclusions: The mPI regimen is a potential effective treatment for economically combating both PHT-sensitive and resistant head lice.

KEYWORDS

head lice, ivermectin, *kdr* mutation, phenothrin, pyrethroid resistance

1 | INTRODUCTION

Head lice are transmitted by head-to-head contact and are more common among young children[1]; 90% of patients with head lice are children aged 0–11 years[2]. The head lice become larvae after the eggs hatch in about 7–12 days, and after 3 molts, they become adults in about 2 weeks[3]. Females live up to 30 days and are expected to lay about 100–200 eggs during their lifetime[4]. Recently, the spread of pyrethroid-resistant head lice has increased in Western countries[4-10] and is also becoming a problem in Okinawa, Japan. Head lice are treated with many types of topical drugs, including the pyrethroid series compound permethrin, ivermectin, spinosad, and dimethicone[11]. Currently, the only treatment for head lice in Japan is pyrethroid series 0.4% phenothrin, which is an over-the-counter (OTC) drug.

Pyrethroid resistance has been associated with amino acid substitutions M815I, T917I, and L920F in the knockdown (*kdr*) gene encoding the α-subunit of voltage-sensitive sodium channel (VSSC)[12]. Six new *kdr* mutations (P813H, I927F, L928A, R929V, L930M, and L932M) were reported in 2017[13]. A recent study also revealed ivermectin-resistant head lice with amino acid substitution mutations in the gene encoding glutamate-gated chloride receptor (GluCl)[14]. In a previous study, the efficacy and safety of a combination regimen comprising 5% phenothrin lotion (Sumithrin Lotion 5%, Kracie Inc; PHT)[15] and 0.5% ivermectin lotion (Sklice lotion 0.5%, Arbor Pharmaceuticals Inc; IVM)[16], designated as a PI regimen, was investigated in head lice patients in Okinawa, Japan. The PHT formulation, which has a 12.5-fold higher concentration of phenothrin than the OTC drug, was approved in 2014 as a scabies treatment for medical use in Japan, and the IVM formulation was approved in 2011 for pyrethroid-resistant head lice in the United states. In the Okinawa study, all head lice had *kdr* mutation and PHT were effective against some pyrethroid-resistant head lice, and IVM was significantly effective against PHT-ineffective head lice[17]. Tsukuba City, where we conducted this study, is located on the mainland of Japan and is located 1611 km north of Okinawa, the southernmost island in Japan. Thus, all of the head lice isolated in the Okinawa study had the *kdr* mutation[17], and the efficacy of the regimen in the presence of a mixture of lice with and without *kdr* mutation has not yet been confirmed.

The aim of the present study was to investigate the efficacy of the modified PI (mPI) regimen, which increased the maximum number of PHT treatments in the PI regimen by one, in Tsukuba with a lower mutation rate compared to Okinawa[9] and the correlation of *kdr* mutations at VSSC loci and GluCl-encoding loci with drug resistance.

2 | MATERIALS AND METHODS

2.1 | Medicines

PHT was purchased from Toho Holdings Co., Ltd. IVM was purchased from The Coghlan Group Inc. The import of IVM was approved by Kanto-Shinetsu Regional Bureau of Health and Welfare.

2.2 | Declarations

The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institutions, and it conforms to the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. The name of the committee is the Ethics Review Committee for Medical Research, Tokyo University of Science, and the approval number is 16037. Individual written informed consent was obtained from all patients and/or guardians.

2.3 | Study design

The study was an open-label, uncontrolled, exploratory study and conducted at the Department of Dermatology, University of Tsukuba Hospital (Ibaraki, Japan), in cooperation with the local medical associations and pharmacist associations. The target cases were 12 cases, and the eligibility criteria were patients diagnosed with head lice infestation aged 6 months or older in which 2 or more lice bodies and 5 eggs were observed. Exclusion criteria were as follows: (a) any history of head lice treatment in the prior 4 weeks; (b) a history of allergy to any of the components of ivermectin or phenothrin; or (c) if estimated as ineligible by the investigators. Eligible patients/parents agreed not to use any other treatment for head lice, comb out nits, or cut or chemically treat hair during the study. Patients older than 20 years received a clear explanation of the aims of the study. If the patients were younger than 20 years, the investigator explained the regimen to patients in an easy-to-understand manner.

Patient IDs were assigned in the order of meeting the eligibility criteria. Patients received sufficient explanation about PHT, focusing on the following: (a) It is approved as a scabies medicine; (b) the ingredient is the same as found in 0.4% Sumithrin shampoo,[18] with the concentration being about 12 times higher; and (c) the application is limited to the head unlike whole-body application for scabies. Similarly, patients received adequate explanation about IVM, focusing on the following: (a) The formulation is not yet approved in Japan but is an approved and prescribed head lice therapeutic in the United States and (b) safety and efficacy are both unknown due to its first use in Japanese subjects. To assess any influence on efficacy, investigators confirmed the use of concomitant medications and the patient's hair type. Hair length was recorded as “short” when above the shoulder, “long” when below the shoulder, and “very long” when below the waist. Hair texture was considered “dry” when the moisture content was low, “oily” when greasy, and “normal” if neither. Hair shape was confirmed as “straight” or “curly”.

The scheme of the mPI regimen is illustrated in Figure 1. The determination of the second medicine received (PHT or IVM) was assessed in the same way as in the Okinawa study[17]. In brief, the medicine to be applied on the second visit (day 8) was determined after collecting head lice 20 times with a comb. If the body of head lice was not found, PHT was used two more times (PHT × 3 group), and if more than one body was found, PHT was switched to IVM

and used once (PHT-IVM group). The investigator followed up each treatment and collected head lice bodies and eggs at the fifth visit on day 29. If lice bodies were found on day 29, IVM was applied once, even in the PHT × 3 group, to confirm the disappearance of lice bodies after one week.

On day 1 (before the first treatment), patients' blood was collected in both groups, on day 8 (before the IVM treatment) and on day 15 in the PHT-IVM group, and on day 22 in the PHT × 3 group, for biochemical blood tests (optional). The investigators submitted data to the clinical study electronic data capture software (OpenClinica, LLC) customized for the study.

2.4 | Evaluation of efficacy and safety

Efficacy was evaluated by the number of collected lice bodies on days 1, 8, 15, 22, and 29. The investigators collected head lice bodies and eggs from the patients' hair and scalp via 20 comb strokes using LiceMeisterComb (The National Pediculosis Association Inc) on a whiteboard (297 × 420 mm), pasted with an adhesive tape in the same way described for the Okinawa study[17,19]. The presence or absence of head lice bodies on the board determined the efficacy of the treatment. If a head lice body was not observed, the investigator reported the treatment as effective. If the body was found, the investigator reported the treatment as ineffective. After the final visit, the whiteboards were sent to the study's data managers at the Tokyo University of Science, who confirmed the number of bodies and counted eggs of head lice. All eggs were examined microscopically (×200) to see whether they were before hatching. Then, the nonempty eggs were counted as "eggs". The visual analogue scale (0: do not feel it, 5: feel it the strongest) was used to assess the itch grade for evaluating itching.

Adverse events involving the skin and scalp were evaluated on days 1 (baseline), 8, 15, 22, and 29, and biochemical blood tests were performed to assess aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels on days 1 (baseline for both groups), 8 (PHT-IVM group), 15 (PHT-IVM group), and 22 (PHT × 3 group). In the Okinawa study, there was no problem in the blood test of all. Therefore, the blood test was not mandatory in this study. Investigators assessed the adverse events (skin, scalp, and ocular irritation), reported by the patients or observed by the investigators, and evaluated the correlation between the adverse events and PHT or IVM. The assessed adverse events were scored using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The study was discontinued if an adverse event was scored 3 or higher.

2.5 | End points

The primary end point was the rate of patients free from lice bodies on day 29. The secondary end point was the difference in the number of lice bodies and eggs collected and the itch grade on days 15, 22, and 29 compared with those at baseline and the reduction rate of bodies and eggs.

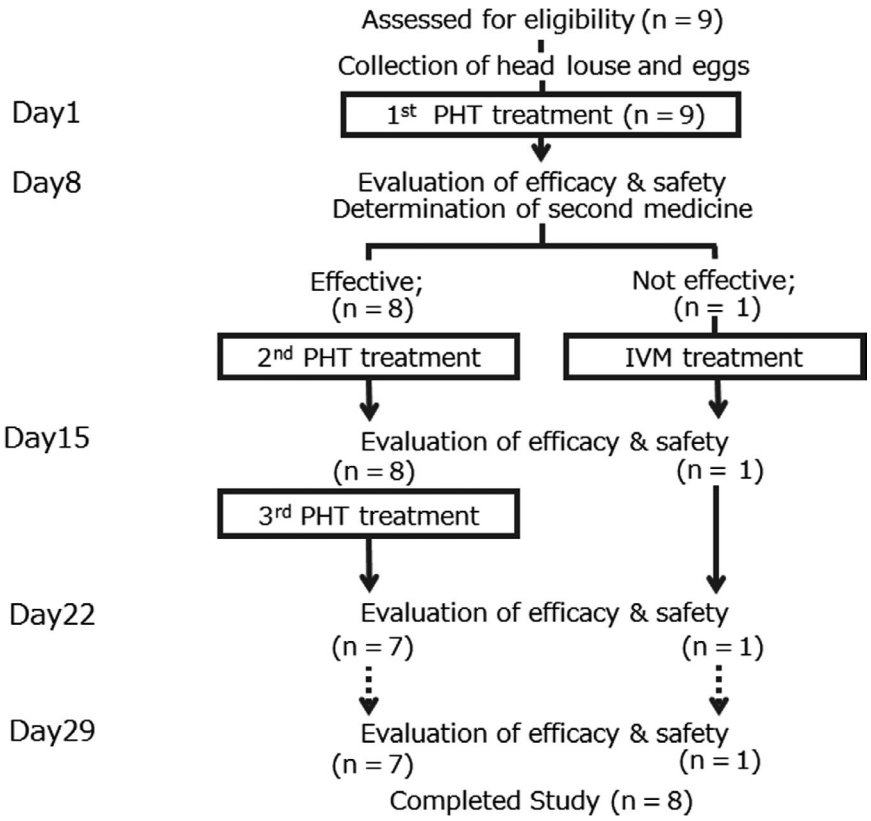

FIGURE 1 Schematic of mPI regimen. PHT, Sumithrin® Lotion 5% (containing 5% phenothrin); IVM, Sklice® Lotion 0.5% (containing 0.5% ivermectin)

TABLE 1 Sequence of the primers for the PCR and cycle sequencing

Primer name	Direction	Sequence (5′→3′)	Use for
5′HL-QS	Forward	ATTTTGCGTTTGGGACTGCTGTT	PCR
3′HL-QS	Reverse	CCATCTGGGAAGTTCTTTATCCA	PCR
5′QSMI	Forward	TGTGGCCTTACTTGTATTCGA	Sequencing
3′QSMI	Reverse	CCCCCCGCATTAAAATTAAAT	Sequencing
5′QSTILF	Forward	AAATCGTGGCCAACGTTAAA	Sequencing
3′QSTILF	Reverse	TTACCCGTGTAATTTTTTCCA	Sequencing
GluCl-7203F	Forward	GCATCATTGCCACCGGTA	Sequencing
GluCl-7750R	Reverse	CAATCGAATTAATTATCTTCCGT	Sequencing
GluCl-7800R	Reverse	GATTGATTTACCAACGACGGC	Sequencing

2.6 | Genotyping of head lice

To determine the presence or absence of *kdr* mutation in VSSC in each head louse, DNA sequencing analysis was performed using the genomic DNA extracted from the head lice bodies or eggs collected on days 1, 8, 15, 22, and 29 as described previously[17]. We analyzed a total of 9 loci for amino acid substitutions, including 3 loci (M815I, P917I, and L920F) investigated in the Okinawa study[17] and an additional 6 loci (P813H, I927F, L928A, R929V, L930M, and L932M).

The genomic DNA was extracted from the lice bodies and eggs using the MightyPrep reagent for DNA (Takara Bio Inc) according to the manufacturer's instructions. The lice were homogenized in sterilized ultrapure water and then used for extraction since they were not lysed in the extraction reagents based on the instructions. The extracted DNA was stored at 4°C until the start of the polymerase chain reaction (PCR) assay. The sequence of the *kdr* mutation of VSSC in head lice was determined according to a previous report[21] with slight modifications. A 908-bp region containing the targeted sites was amplified by PCR using 5′HL-QS and 3′HL-QS primers (Table 1). Forty PCR cycles were performed using MightyAmp DNA Polymerase Ver.3 (Takara Bio Inc) and Ez Cycler EZC-96 (Iwaki Co., Ltd.) under the following conditions: denaturation at 98°C for 10 s, annealing at 63°C for 15 s, and extension at 68°C for 60 s. The PCR products were mixed with the primers for 5′QSMI, 3′QSMI, 5′QSTILF, or 3′QSTILF (Table 1), and then subjected to sequence analysis by DNA Sequence Analysis Service (Fasmac Co., Ltd.).

The mutations at T236A, A251V, or H272R in *GluCl* were analyzed by PCR of the genomic DNA using the primers 7203F and 7800R (Table 1) with the same condition as described above for the *kdr* mutation analysis and then mixed with the primer 7750R (Table 1) for the sequencing analysis. The primers for the *GluCl* mutation analysis were designed based on a previous report[14].

2.7 | Statistical analysis

Steel's multiple comparison Wilcoxon test was performed to investigate the significance of the changes in the number of bodies and eggs and the itching grade on days 15, 22, and 29 from baseline (before day 1 of administration).

3 | RESULTS

3.1 | Patients

From July 2017 to March 2019, 11 patients were enrolled in this study from a nearby clinic; patients with ID 1 and 2 did not meet the eligibility criteria, leaving a total of 9 patients enrolled. Eight patients received all treatments and underwent all evaluations. Patient with ID 11 dropped out of the study on day 8 because the eggs were removed when her family used a comb. The background profile of the 8 patients and the single-dose treatments received on days 1, 8, and 15 are shown in Table 2. On day 8, 1 week after the first PHT treatment, PHT was effective in 7 of the 8 patients (ID 3, 4, 5, 7, 8, 9, and 10) and was therefore continued for these patients (PHT × 3 group). For the remaining patient in which PHT was ineffective (ID 6), the treatment was switched to IVM (PHT-IVM group).

3.2 | Efficacy

Table 3 shows the numbers of lice bodies and eggs collected from the patients and the grade of itch during each visit. On day 8, seven (ID 3, 4, 5, 7, 8, 9, and 10) of the eight patients were free of head lice bodies and received a double dose of PHT. Since only Patient 6 had a head lice body detected at this point, this patient received IVM. On day 22, all seven patients (ID 3, 4, 5, 7, 8, 9, and 10) receiving PHT were head lice-free. In addition, ID 6 receiving IVM was head lice-free on day 22. At follow-up on day 29, all eight patients were still free of head lice bodies. Therefore, the rate of patients who were lice-free with the mPI regimen was 100% on both days 22 and 29. There was a significant reduction in the average number of bodies ($p < 0.001$ and $p < 0.001$) and itching grade ($p = 0.005$ and $p < 0.001$) from baseline to day 22 and to day 29, respectively. The reduction of the number of eggs was not significant on day 22 ($p = 0.16$) but was on day 29 ($p = 0.005$) on day 29 from the baseline.

3.3 | Safety

In ID 3, eczema was observed on both of the lower limbs 3 days before the fifth visit (day 29). Since the site was not close to the head,

TABLE 2 Patient characteristics and dose of PHT and IVM

							Hair type			Single dose of treatment					
							Length	Texture	Shape	Dose(g)			Dose/BSA(g/m^2)		
										Day 1	Day 8	Day 15	Day 1	Day 8	Day 15
Group	ID	Sex	Age	Height (cm)	BW (kg)	BSA (m^2)[a]	Very long → Very short	Dry/Normal/ Oily	Straight/ Curly	PHT	PHT/ IVM	PHT	PHT	PHT/ IVM	PHT
PHT × 3	3	F	9	125	25	0.9	Long	Normal	Straight	60	60	60	65	65	65
	4	M	7	114	20	0.8	Very short	Normal	Straight	30	30	30	38	38	38
	5	F	41	153	49	1.4	Very short	Dry	Straight	60	60	60	42	42	42
	7	F	44	155	50	1.5	Long	Normal	Curly	90	90	90	61	61	61
	8	F	34	160	53	1.5	Short	Dry	Straight	60	60	60	39	39	39
	9	M	9	139	36	1.2	Short	Normal	Straight	60	60	60	51	51	51
	10	F	7	124	22	0.9	Very long	Normal	Straight	90	90	90	102	102	102
	Ave or Ratio		21.6	138.6	36.4	1.2	1:2:2:2	2:5:0	6:1	64.3	64.3	64.3	56.9	56.9	56.9
	SD		17.2	18.0	14.3	0.3				20.7	20.7	20.7	22.5	22.5	22.5
PHT → IVM	6	F	9	140	31	1.1	Short	Normal	Curly	60	51	—	54	46	—
Total	Ave or Ratio		20.0	138.8	35.8	1.2	1:2:3:2	2:6:0	6:2	63.8	62.6	64.3	56.5	55.5	56.9
	SD		16.5	16.6	13.4	0.3				19.2	19.7	20.7	20.9	21.2	22.5

Note: PHT, Sumithrin® Lotion 5% (containing 5% phenothrin); IVM, Sklice® Lotion 0.5% (containing 0.5% ivermectin).

[a]BSA is the abbreviation for body surface area calculated using the formula Du Bois.

TABLE 3 Number of lice (bodies and eggs) and grade of itching on each day

		Number of lice bodies					Number of eggs					Grade of itching				
		Day					Day					Day				
Group	ID	1	8	15	22	29	1	8	15	22	29	1	8	15	22	29
PHT × 3	3	2	0	0	0	0	63	6	1	2	3	4	0	0	0	0
	4	5	0	0	0	0	29	3	2	0	1	1	0	0	0	0
	5	2	0	0	0	0	5	0	1	0	0	1	0	0	0	0
	7	2	0	0	0	0	59	3	5	2	1	1	0	0	1	0
	8	2	0	0	0	0	15	7	3	3	2	3	0	0	0	0
	9	10	0	0	0	0	8	45	21	79	6	3	0	0	0	0
	10	2	0	0	0	0	30	4	11	57	0	3	0	0	0	0
	Ave	3.6	0.0	0.0	0.0	0.0	29.9	9.7	6.3	20.4	1.9	2.3	0.0	0.0	0.1	0.0
	SD	3.0	0.0	0.0	0.0	0.0	23.3	15.7	7.4	33.1	2.1	1.3	0.0	0.0	0.4	0.0
PHT → IVM	6	22	1	0	0	0	792	142	37	15	6	1	1	0	1	0
Total	Ave	5.9	0.1	0*	0*	0*	125.1	26.3	10.2	19.8	2.4*	2.1	0.1	0*	0.3*	0*
	SD	7.1	0.4	0.0	0.0	0.0	270.3	49.0	12.8	30.7	2.4	1.2	0.4	0.0	0.5	0.0

Note: PHT, Sumithrin® Lotion 5% (containing 5% phenothrin); IVM, Sklice® Lotion 0.5% (containing 0.5% ivermectin).

*Significant different compared to day 1 (baseline) ($p < 0.05$).

a causal relationship between eczema and PHT was ruled out. On day 22, AST and ALT levels were 25 and 10 IU/μL, respectively, with no abnormal liver function observed for ID 6 who was able to cooperate. In ID 11, after the second application of PHT on day 8, mild erythema appeared on the application area and persisted for 2 days, which was diagnosed as contact dermatitis (Grade 1 in CTCAE Ver.4.0 SOC). Although the causal correlation of PHT could not be ruled out, the erythema recovered at day 15. No other PHT- or IVM-related adverse events were observed.

3.4 | Genotyping of head lice

The genotype on *kdr* mutations in VSSC was analyzed for 123 samples; up to 5 samples were subjected to the analysis per patient per day. Of the 123 samples, the test for 21 samples failed because of unsatisfactory quality of the preserved DNA. The remaining 102 samples were classified into two types, with and without *kdr*-type mutation at the three locations of M815I–L920F, and the additional six sites (P813H and I927F–L932M) were mutated in all samples (Table 4). The *kdr*-type mutation at M815I–L920F was detected in 19.6% (20/102) of the samples collected in this study. The ratio of mutants at M815I–L920F was 10.8% (9/83) in samples from patients (ID 3–5 and 7–10) who were sensitive to the first PHT application, but was higher at 57.9% (11/19) in samples from the patient (ID 6) for whom the first PHT was ineffective. Therefore, the mutation rate more than doubled in head lice collected from the patient for whom PHT was ineffective. In addition to the previous Okinawa study[17], two new haplotypes for VSSC loci of head lice were identified in Tsukuba. The four haplotypes observed in Okinawa and Tsukuba (Figure 2) are new as they have not reported in any other previous studies.

The T236A, A251V, or H272R genotypes in the *GluCl* loci were successfully analyzed for 75 samples (2–13 samples per patient). All 75 samples were nonmutated at these three sites (Table S1).

4 | DISCUSSION

In this study, the efficacy and safety of the mPI regimen were evaluated, which comprises PHT and IVM. Since the PHT formulation provided in this study contains a 12.5-fold higher concentration than the OTC PHT formulation for head lice, it could have potential efficacy against pyrethroid-resistant head lice. No lice bodies were found in any of the patients at day 29 after treatment with the mPI regimen (Table 2, $n = 8$), indicating that the regimen was effective against head lice. The rate of patients who were lice-free with the mPI regimen was 100%, which is better than that reported in the previous Okinawa study (92%). The effects of the mPI regimen in Tsukuba and the PI regimen in Okinawa could not be compared directly, since the population of pyrethroid-resistant head lice was extremely different and the sample size in both studies was very small. With respect to adverse events, only one patient in the Tsukuba study had a mild skin rash after PHT treatment, and there were no serious adverse events in the present Tsukuba or in the previous Okinawa study. Although eye irritation was reported during an IVM clinical trial in the United states, this could also be due to the use of goggles in this study[19].

The efficacy of these treatments was evaluated by the presence or absence of bodies of lice as in the IVM clinical trial[19], but it is also necessary to consider the presence of eggs. The head lice bodies were disappeared in all patients during the observation period, although eggs were still present in 75% of patients on day 29 (Table 3).

TABLE 4 Number of *kdr*-type mutated head lice and eggs, and mutation rate

Group	ID	M815I					T917I					L920F					Mutation rate
		Day					Day					Day					
		1	8	15	22	29	1	8	15	22	29	1	8	15	22	29	
PHT × 3 ($n = 7$)	3	0/2	0/5	–	0/2	1/2	0/2	0/5	–	0/2	1/2	0/2	0/5	–	0/2	1/2	9.1%
	4	0/5	1/3	0/2	–	0/1	0/5	1/3	0/2	–	0/1	0/5	1/3	0/2	–	0/1	9.1%
	5	1/5	–	0/1	–	–	1/5	–	0/1	–	–	1/5	–	0/1	–	–	16.7%
	7	1/2	2/2	1/1	1/1	–	1/2	2/2	1/1	1/1	–	1/2	2/2	1/1	1/1	–	83.3%
	8	0/2	0/5	0/2	0/3	0/2	0/2	0/5	0/2	0/3	0/2	0/2	0/5	0/2	0/3	0/2	0%
	9	0/5	0/5	0/4	0/3	1/3	0/5	0/5	0/4	0/3	1/3	0/5	0/5	0/4	0/3	1/3	5.0%
	10	0/2	0/4	0/4	0/5	–	0/2	0/4	0/4	0/5	–	0/2	0/4	0/4	0/5	–	0%
PHT → IVM ($n = 1$)	6	5/5	1/1	4/5	1/4	0/4	5/5	1/1	4/5	1/4	1/4	5/5	1/1	4/5	1/4	1/4	57.9%

Note: Each number shows the ratio of mutated sample to its analyzed sample.

In this study, we confirmed that all eggs were prehatched, but it is impossible to determine whether the eggs were actually alive. In ID 9 and 10 who were family members and were being treated at the same time, increases in egg count of over 50 were observed at day 22. The increase may have been due to reinfection in public spaces such as school, but this was not confirmed. More observational data are needed to conclude whether eggs should be observed until disappearance as a measure of continuity of infection.

Head lice in seven patients (ID 3, 4, 5, 7, 8, 9, and 10) were treated thrice with PHT and were eradicated. Since only a fraction of the lice from Patients 5 and 7 had a *kdr* mutation, PHT could exterminate them. Similarly, PHT was effective in a fraction of patients infected with head lice carrying the *kdr* mutation in the Okinawa study. This result might suggest that high concentrations of PHT overcome the pyrethroid resistance due to *kdr* mutations. The treatment success rate in the PHT-only treatment group receiving the mPI regimen in Tsukuba (100%) was higher than that in the PI regimen in Okinawa (92%). This increase might be due to the increase in the number of therapeutic interventions from two in the PI regimen to three in the mPI regimen. We concluded that the third application of PHT was necessary on day 15 when the surviving eggs hatched. The international guidelines for clinical studies on the efficacy and safety of pediculicides recommend a protocol with three therapeutic interventions that takes into account the timing of egg hatching[20].

PHT was ineffective in only one case (ID 6), and IVM was applied at day 8. Although no head lice bodies were found until day 29 after then, the eggs had not disappeared until day 29.

Regarding the positioning of pyrethroid drugs and IVM in international guidelines for head lice treatment, in European guidelines, a 1% permethrin cream is recommended as the first line therapy, 0.2% phenothrin lotion is recommended as the second line therapy, and topical IVM is recommended as miscellaneous therapy, which was reported as effective and generally well-tolerated for pediculosis pubis (level of evidence IV; grade C recommendation)[22]. In the CADTH review, an independent, not-for-profit organization responsible for providing data to healthcare decision-makers, topical IVM was not mentioned, as no randomized controlled trials have been conducted comparing it with pediculicides[23]. IVM is positioned as an alternative therapy in the guidelines, since there is no mention of IVM utilization depending on the pyrethroid resistance status. Since the emergence of IVM-resistant head lice was recently reported in Senegal, it is necessary to focus on the proper use of IVM[14].

Head lice treatment requires predicting the effective treatment for each patient, using pyrethroid and the alternative IVM properly. Head lice genotyping is therefore an ingenious method to predict the individual response to PHT. However, the phenotype (response to PHT) does not always match the genotype (*kdr* mutation), and gene sequencing is time-consuming. In the PI and mPI regimens, the first PHT treatment is used to investigate the response to PHT. This in vivo phenotyping could be one of the best methods to choose an effective treatment for head lice.

In addition, drug cost is an important factor in drug selection. The cost of PHT is 4436 yen/average single dose (64.1 g), and the

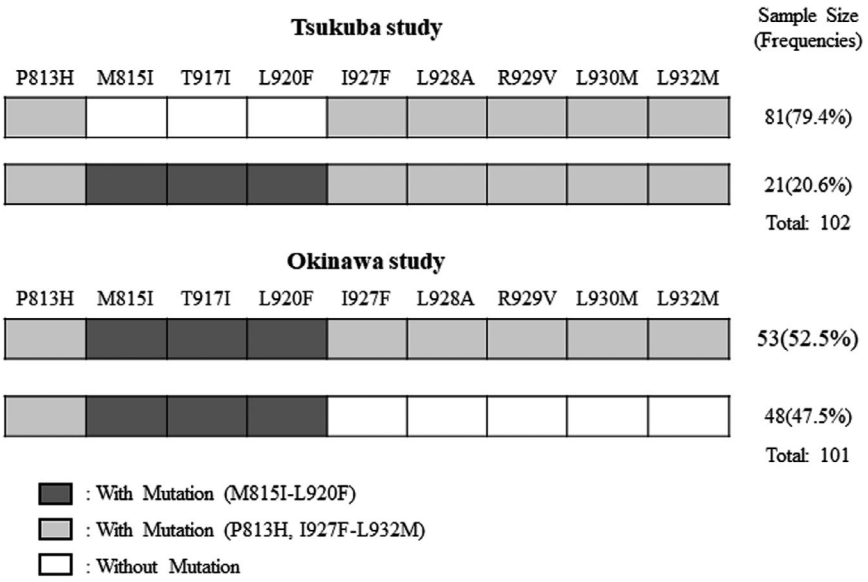

FIGURE 2 Comparison of the mutation location of head lice

cost of overall treatment is 13,308 yen. The cost of IVM is 17,436 yen/average single dose (51.0 g), which is also the cost for the whole treatment. In short, the efficacy of PHT against pyrethroid-resistant lice may be lower than that of IVM, but PHT is less costly.

Since its first report in France in 1994, pyrethroid-resistant lice has become a serious issue[24]. Previous research in the United States suggested that the increasing frequency of the presence of *kdr*-type mutations in head louse populations coincides with clinical failures reported in controlled studies[25]. Previously, *kdr* mutations were found in louse samples from patients in whom pyrethroids were ineffective[10]. Studies have also shown that only a fraction of the three mutations, especially the T917I mutation, may be critical for pyrethroid resistance expression[10,21,26,27]. The *kdr* mutation has a geographic origin that explains the regional variance in the incidence of pyrethroid resistance[28]. In 2009, the frequency of resistance was 96% in Okinawa and was 5% in the mainland areas of Japan[5,9]. The ratio of lice with M815I-L920F mutations was 100% in the Okinawa study[17], whereas it was 19.6% in the present Tsukuba study. Furthermore, since the resistance rate has increased in both Okinawa and the mainland, the spread of pyrethroid-resistant mutants throughout Japan is concerning.

The genotyping in the Okinawa study[17] and the present study showed new types of head lice mutants. All of the louse samples in the Okinawa study carried the four mutations P813H, M815I, T917I, and L920F with or without the I927F–L932M mutations[17], whereas all samples in the present study had the P813H and I927F–L932M mutations, which appeared independently of pyrethroid resistance (Figure 2). Of these mutations, P813H, I927F, and L932M have been only reported in the body lice[13]. Data collected in Okinawa[17] and from the present study revealed the presence of three mutations in head lice for the first time. Although further investigations are needed to clarify the correlation of mutants and haplotypes with drug resistance, these findings will contribute to developing more effective therapeutics against head lice and tracking their geographic transmission.

In the present study, we additionally analyzed the *GluCl* mutation, which may be associated with IVM head lice resistance, and found no T236A, A251V, or H272R mutant at three out of five sites reported in the Senegal study[14]. IVM-resistant head lice are not currently reported in Japan; however, the potential of transmission and expansion may require attention.

Overall, our results demonstrate that the mPI regimen is a potential effective treatment for economically combating both PHT-sensitive and resistant head lice. This study revealed four new haplotypes in *kdr* loci that may contribute to future studies for the development of effective therapeutics against head lice.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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