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Hd1 function conversion in regulating heading is dependent on gene combinations of *Ghd7*, *Ghd8*, and *Ghd7.1* under long-day conditions in rice

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Abstract *Ghd7*, *Ghd8*, *Ghd7.1*, and *Hd1* are all pleiotropic genes regulating heading date, plant height, and yield in rice. Early studies showed that *Hd1* promoted heading under short-day conditions (SD) and delayed heading under long-day conditions (LD). Recent studies found that *Hd1* also promoted heading in some genetic backgrounds under LD. In this study, we developed a series of near-isogenic lines for *Ghd7*, *Ghd8*, *Ghd7.1*, and *Hd1* in the Zhenshan 97 (ZS97) and Minghui 63 (MH63) backgrounds and recorded their heading dates. In the ZS97 background, *Ghd7* alone triggered the conversion of *Hd1* function from promoting to suppressing heading under LD. *Ghd8* alone and *Ghd7.1* alone did not promote but enhanced *Ghd7*-mediated *Hd1* function conversion. In the MH63 background, *Ghd7* alone and *Ghd7.1* alone did not convert *Hd1* function under LD, but they jointly promoted *Hd1* function conversion. Conversion of *Hd1* function occurred only under LD but not SD. Transcript analysis showed that downregulation or upregulation of *Ehd1* and *Hd3a* by *Hd1* in the lines under LD was determined by genetic background. In summary, multiple gene combinations of *Ghd7*, *Ghd7.1*, and *Ghd8* and unknown genes caused conversion of *Hd1* function. Moreover, the different gene combinations had a big difference in photoperiod sensitivities. These findings lay a solid foundation for further unveiling the molecular mechanism of *Hd1* function conversion and provide guidance for heading date improvement in rice.

Keywords Gene combinations · Long-day conditions · Heading date · *Hd1* function conversion · Photoperiod sensitivity

Zhanyi Zhang and Bo Zhang contributed equally to this work.

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Introduction

Rice (*Oryza sativa* L.) is an important cereal crop that is cultivated across the world. Environmental factors such as photoperiod, temperature, rainwater, and illuminance in rice planting regions vary temporally and geographically. To gain high yield, rice cultivars must avoid unfavorable growth conditions during its life cycle and adequately utilize light energy and temperature resources. Thus, flowering at a suitable time is essential to rice production.

Rice is a short-day plant whose flowering is promoted under short-day conditions (SD), while it is

delayed under long-day conditions (LD). The photoperiod pathway is an important way to regulate heading in rice. *Heading date 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*), orthologs to *FLOWERING LOCUS T* (*FT*) of Arabidopsis, are florigen genes in rice that switch on flowering (Komiya et al. 2008; Komiya et al. 2009; Taoka et al. 2011). Two independent pathways are mediated by *Heading Data 1* (*Hd1*) and *Early heading date 1* (*Ehd1*) to regulate *Hd3a* and *RFT1* expression. *Hd1*, an ortholog of CONSTANS in Arabidopsis, delays and promotes flowering by downregulating and upregulating the expression of *Hd3a* and *RFT1* under LD and SD, respectively (Yano et al. 2000). *Ehd1*, encoding a B-type response regulator, functions as a homodimer and induces heading by upregulating *Hd3a* and *RFT1* under both LD and SD (Doi et al. 2004; Cho et al. 2016). *Ghd7*, *Ghd8*, and *Ghd7.1* repress flowering by inhibiting *Ehd1* expression under LD (Xue et al. 2008; Yan et al. 2011; Hori et al. 2013). However, recent studies have shown that *Hd1* also regulates *Ehd1* expression (Nemoto et al. 2016; Zhang et al. 2017), which indicates that the two pathways are tightly associated.

Genetic interactions between *Ghd7*, *Ghd8*, *Ghd7.1*, and *Hd1* regulate rice flowering (Lin et al. 2000; Shibaya et al. 2011; Zhang et al. 2015). *Hd1*, *Ghd7*, and *Ghd7.1* all encode CO, CO-like, and TOC1 (CCT)-domain proteins (Yano et al. 2000; Xue et al. 2008; Hori et al. 2013; Liu et al. 2013; Zhang et al. 2017), while *Ghd8* encodes a CCAAT-box-binding transcription factor protein, HAP3H (Yan et al. 2011). Hd1 forms complexes with Ghd7 or Ghd8 to repress flowering by downregulating *Ehd1* and *Hd3a* under LD (Nemoto et al. 2016; Du et al. 2017; Goretti et al. 2017; Zhu et al. 2017). Ghd8 interacts with Ghd7 and Ghd7.1 in yeast (Goretti et al. 2017). These results imply that *Ghd7*, *Ghd8*, *Hd1*, and *Ghd7.1* interactively regulate flowering. However, the detailed genetic interactions and molecular mechanisms which they regulate flowering are not very clear.

Recent studies indicated that *Hd1* constantly promoted heading date and exhibited photoperiod insensitivity under both SD and LD on a genetic background without *Ghd7* or *Ghd8* (Du et al. 2017; Zhang et al. 2017), which conflicted with earlier findings (Yano et al. 2000). The phenomenon that *Hd1* can switch from its presumed function of promoting heading to repressing heading under LD was termed *Hd1* function conversion. Why *Hd1* function conversion occurs has been controversial. One study insisted that *Ghd7* was required for the conversion (Zhang et al. 2017), while another study deemed that *Ghd8* rather than *Ghd7* was required for its conversion (Du et al. 2017).

To resolve the inconsistent results, the heading dates of a series of near-isogenic lines (NILs) of *Ghd7*, *Ghd8*, and *Hd1* in the Zhenshan 97 (ZS97) background were investigated in this study. A series of NILs of *Ghd7*, *Ghd7.1*, *Hd1*, and *Ghd8* on either the ZS97 or the Minghui 63 (MH63) background was also used. We found that *Ghd8* converted *Hd1* function depending on *Ghd7*, and *Ghd7.1* also participated in *Hd1* function conversion together with *Ghd7* or *Ghd8*. By surveying the photoperiod sensitivity (PS) of all these NILs, we found that different combinations of *Ghd7*, *Ghd7.1*, *Hd1*, and *Ghd8* had diverse PS. This study sheds some light in the molecular mechanism of *Hd1* function conversion.

Materials and methods

Plant materials

Zhenshan 97 (*ghd7ghd8ghd7.1Hd1*) is a cultivar with nonfunctional *Ghd7*, *Ghd8*, and *Ghd7.1* and functional *Hd1* (Xue et al. 2008; Yan et al. 2011, 2013; Zhang et al. 2017). In a previous study, ZS-G7G8H1 (*Ghd7Ghd8ghd7.1Hd1*), a near-isogenic line (NIL) with introgressed functional *Ghd7* and *Ghd8* from MH63 and 93-11, respectively, was developed in the ZS97 background (Zhang et al. 2015). ZS-g7g8h1 (*ghd7ghd8ghd7.1hd1*) was a NIL introgressed with nonfunctional *hd1* from Teqing (TQ) into the ZS97 background (Zhang et al. 2017). We obtained two F_1 hybrid plants by crossing ZS-G7G8H1 with ZS-g7g8h1 and then developed two F_2 populations with *Ghd7*, *Hd1*, and *Ghd8* segregating by self-pollination. Eight homozygous combinations of *Ghd7*, *Ghd8*, and *Hd1,* namely, ZS-G7G8H1, ZS-G7G8h1, ZS-G7g8H1, ZS-G7g8h1, ZS-g7G8H1, ZS-g7G8h1, ZS-g7g8H1, and ZS-g7g8h1, were identified from these two populations by functional markers or closely linked markers (Supplementary Table 1) and selfed for many seeds for subsequent experiments (Supplementary Fig. 1A).

ZS-g7G7.1H1 (*ghd7ghd8Ghd7.1Hd1*) was the NIL introgressed with functional *Ghd7.1* form TQ in the

ZS97 background (Liu et al. 2013). It was crossed with ZS-g7g8h1 to generate an F_2 population in the ZS97 background and ZS-g7G7.1H1, ZS-g7G7.1h1, ZS-g7g7.1H1, and ZS-g7 g7.1 h1 were identified from this population (Supplementary Fig. 1B). Then, ZS-g7G7.1H1 was crossed with ZS-G7g8h1 to obtain the ZS-G7G7.1H1, ZS-G7G7.1h1, ZS-G7g7.1H1, and ZS-G7g7.1h1 lines (Supplementary Fig. 1C). The NIL ZS-G7G8hd1 was crossed with ZS-g7G7.1H1, an F_2 population was planted, and the homozygous genotypes ZS-g7G8G7.1H1, ZS-g7G8G7.1h1, ZS-G7G8G7.1H1, and ZS-G7G8G7.1h1 were identified from this population (Supplementary Fig. 1D).

MH63 (*Ghd7ghd8Ghd7.1hd1*) is an indica cultivar containing nonfunctional *Ghd8* and *Hd1* and functional *Ghd7* and *Ghd7.1* (Yan et al. 2013; Zhang et al. 2015; Zhang et al. 2017). MH-G7G7.1H1 (*Ghd7ghd8Ghd7.1Hd1*) and MH-G7g7.1h1 (*Ghd7ghd8ghd7.1hd1*) introgressed with functional *Hd1* and nonfunctional *ghd7.1* in the MH63 background, respectively, were identified from advanced chromosome segment substitution lines (CSSLs) in the MH63 background with ZS97 as the donor parent (Shen and Xing 2014; Zhang et al. 2017). They were crossed to get an F_2 population in the MH63 background, and four homozygous MH-G7G7.1H1, MH-G7G7.1h1, MH-G7g7.1H1, and MH-G7g7.1h1 were identified (Supplementary Fig. 1E). MH-g7G7.1H1 (*ghd7ghd8Ghd7.1Hd1*) was an MH63-background line that contained nonfunctional *ghd7* and functional *Hd1* (Zhang et al. 2017). It was crossed with MH-G7g7.1h1 to get MH-g7G7.1H1, MH-g7G7.1h1, MH-g7g7.1H1, and MH-g7g7.1h1 homozygous plants (Supplementary Fig. 1F).

Field experiments and photoperiod treatment

Plant materials were sown in the middle of May at the experimental station of Huazhong Agriculture University, Wuhan, Hubei province, China (31°N). The day length of Wuhan was more than 13.5 h from the middle of May to the beginning of August (natural long-day conditions, NLD). Twenty-five-day old seedlings were transplanted to the field with 16.5 cm between plants in a row and 26.5 cm between rows. Plant materials were sown in the beginning of December at Lingshui, Hainan province, China (18°N). The average day length was less than 12 h from December to March in Lingshui (natural short-day condition, NSD). Thirty-day-old seedlings were transplanted to the field with the same planting density as was used in Wuhan.

For photoperiod treatment, five 20-day-old seedlings of each genotype were transplanted to the field at a density of approximately 10 cm × 10 cm in Wuhan. The plants were grown under SD with a day length of 8 h and darkness of 16 h by shading treatment until all plants headed. At the same time, another five 20-day-old seedlings of each genotype were simultaneously grown in the same field under NLD for LD.

Identification of the genotypes of *Ghd7*, *Ghd8*, *Ghd7.1*, and *Hd1*

The DNA of plant materials was extracted from leaves using the CTAB method (Stewart Jr. and Via 1993). Then, the linked markers MRG4436, Z9M, S56, and Indel37 were used to identify the genotypes at *Ghd7*, *Ghd8*, *Hd1*, and *Ghd7.1*, respectively. The primers of these markers are available in Supplementary Table 1.

Expression analysis of *Ehd1* and *Hd3a* by quantitative real-time PCR

To detect *Ehd1* and *Hd3a* expression in the series of NILs, the second leaves were collected from three plants of each genotype at 40 days old at 8:00 under NLD as biological replicates. A 100-mg sample ground with liquid nitrogen was suspended with TransZol (TransGen Biotech, China) reagent to extract RNA. Then, 3 μg of RNA treated with DNase I was used to synthesize cDNA by using reverse transcriptase M-MLV reagent (Invitrogen). Relative expression levels of *Ehd1* and *Hd3a* were analyzed by quantitative real-time PCR conducted with an ABI PRISM 7900 Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. The primers used are listed in Supplementary Table 1.

Data analysis

The Student's *t* test was used for pairwise comparison. Multiple comparisons were made using Duncan's test (Duncan 1955). The photoperiod sensitivity (PS) index was measured using the averages of the heading date under LD, subtracting the averages of the heading date under SD.

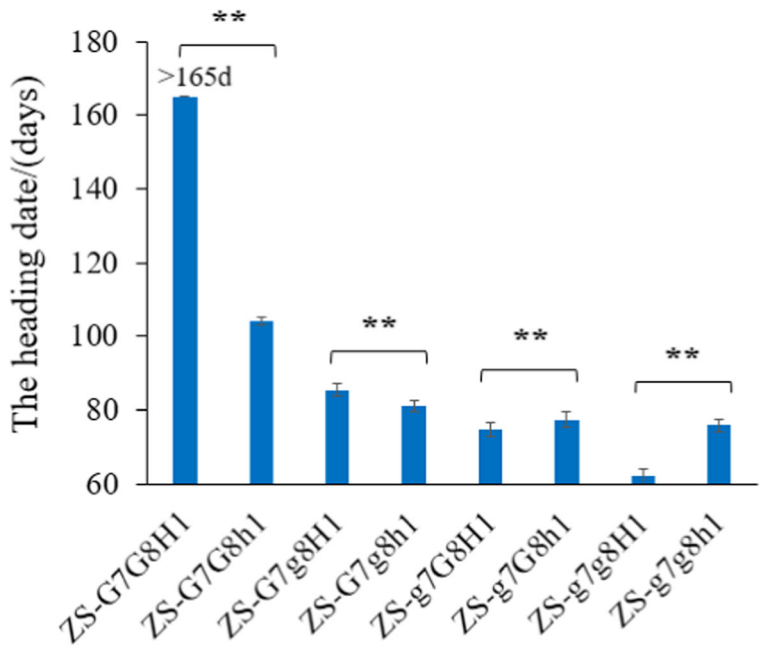

Fig. 1 The heading date of a series of *Ghd7*, *Ghd8* and *Hd1* NILs in the ZS97 background under NLD. "> 165 d," the heading date was more than 165 days, beyond our survey. "**," significant difference in heading date at $P < 0.01$ by Student's *t* test.

Results

Hd1 function conversion depends on *Ghd7* in the ZS97 background

To study the roles of *Ghd7* and *Ghd8* in *Hd1* function conversion, eight homozygous three-gene combinations of *Ghd7*, *Ghd8*, and *Hd1* were selected from two NIL-F_2 populations to survey the heading date under NLD. ZS-g7g8H1 and ZS-g7G8H1 flowered 9.7–13.9 and 2.7–8.6 days earlier compared to ZS-g7g8h1 and ZS-g7G8h1, respectively (Fig. 1; Supplementary Table 2). Thus, *Hd1* promoted heading without *Ghd7* and *Ghd8*, or only with *Ghd8* in ZS97. However, ZS-G7g8H1 and ZS-G7G8H1 flowered 3.3–5.0 days and > 59.8 days later than

Table 1 The photoperiod sensitivity of a series of three-gene combinations of *Ghd7*, *Ghd8*, and *Hd1*

Genotype	HD (days)		PS	HD (days)
	LD	SD		NSD
ZS-G7G8H1	$> 165^{a}$**	59.4 ± 1.9^{e}	> 100.6	124.6 ± 5.4^{a}
ZS-G7G8h1	102.3 ± 2.1^{b}	101.3 ± 1.0^{a}	1	125.8 ± 2.3^{a}
ZS-G7g8H1	79 ± 1.4^{c}**	58.4 ± 1.3^{e}	20.6	95.4 ± 6.1^{cd}
ZS-G7g8h1	78.3 ± 1.5^{c}	79.8 ± 2.4^{b}	− 1.5	109.3 ± 3.1^{b}
ZS-g7G8H1	73 ± 0.8^{e}**	56.8 ± 2.4^{ef}	16.2	84.2 ± 4.5^{e}
ZS-g7G8h1	74.8 ± 1.0^{d}**	69.0^{d}	5.8	97.0 ± 3.7^{c}
ZS-g7g8H1	57.3 ± 1.5^{f}**	54.3 ± 0.6^{f}	3	83.5 ± 4.2^{e}
ZS-g7g8h1	74 ± 0.8^{de}	71.8 ± 1.6^{c}	2.2	94.3 ± 4.1^{d}

LD long day, *SD* short day, *NSD* natural short day, *PS* photoperiod sensitivity

There was no difference in other environmental factors except day length between LD and SD. Different letters within a column (a, b, c, d, e, and f) indicate a significance difference in multiple comparisons of the same genetic background at $P < 0.05$. **, significant difference in heading date between short-day conditions and long-day conditions at $P < 0.01$ by Student's *t* test

ZS-G7g8h1 and ZS-G7G8h1, respectively (Fig. 1; Supplementary Table 2). This indicated that *Hd1* suppressed heading date with *Ghd7*, or together with *Ghd7Ghd8*, and suggested that *Ghd8* without *Ghd7* did not convert *Hd1* function from promoting to delaying heading date in the ZS97 background, which was not in agreement with the previous report that single *Ghd8* could convert *Hd1* function in the 93-11 background (Du et al. 2017). Unlike *Ghd8*, *Ghd7* alone slightly converted *Hd1* function for 3.3–5 days in the ZS97 background, consistent with our previous study (Zhang et al. 2017). However, *Hd1* significantly delayed heading (more than 59.8 days) and caused failed heading in the normal growing season under the background of functional *Ghd7Ghd8* combination. Taken together, the data showed that *Ghd8* largely enhanced *Ghd7*-mediated *Hd1* function conversion in the ZS97 background.

Photoperiod sensitivity of eight homozygous three-gene combinations of *Ghd7*, *Ghd8*, and *Hd1* in the ZS97 background

Ghd7, *Ghd8*, and *Hd1* are photoperiod-sensitivity genes influencing rice adaptability to different ecological areas. To investigate the photoperiod sensitivity of eight homozygous three-gene combinations and evaluate whether *Hd1* function conversion occurred in short days, these eight homozygous combinations of *Ghd7*, *Ghd8*, and *Hd1* were grown under LD, NSD, and SD. Under SD and NSD, *Hd1* always promoted heading date regardless of genetic backgrounds (Table 1), which suggested that *Hd1* function conversion did not happen under SD conditions.

Estimates of photoperiod sensitivity (PS) showed that ZS-G7G8H1 had the strongest PS of more than 100.6 days, followed by ZS-G7g8H1 and ZS-g7G8H1 of 20.6 days and 16.2 days, respectively (Table 1). *Hd1* was weakly sensitive to photoperiod in ZS-g7g8H1 (PS = 3 days) (Table 1). For the combinations without *Hd1*, their PS was weak or even absent (Table 1). For example, the PS of ZS-g7G8h1 and ZS-G7g8h1 were 5.8 days and − 1.5 days, respectively. It was surprising that ZS-G7G8h1 lost its photoperiod sensitivity and had similar heading dates of 102 and 101 days between NLD and SD, indicating its photoperiod insensitivity (Table 1).

Ghd7.1 enhances *Ghd7*-mediated *Hd1* function conversion in the ZS97 background

Ghd7.1 has similar functions to *Ghd7* under LD conditions (Hori et al. 2013; Liu et al. 2013). We hypothesized that *Ghd7.1* also triggered *Hd1* function conversion. To verify our hypothesis, four homozygous combinations of *Ghd7.1* and *Hd1* in the ZS97 background were used to measure heading date under NLD. Both ZS-g7g7.1H1 and ZS-g7G7.1H1 flowered earlier than ZS-g7g7.1h1 and ZS-g7G7.1h1 for 16.6 and 9 days, respectively (Fig. 2b). Thus, *Ghd7.1* alone did not trigger *Hd1* function conversion in the ZS97 background. We also developed combinations of ZS-G7G7.1H1, ZS-G7G7.1h1, ZS-G7g7.1H1, and ZS-G7g7.1h1. Both ZS-G7G7.1H1 and ZS-G7g7.1H1 headed later than did ZS-G7G7.1h1 and ZS-G7g7.1h1 by 21 and 3.7 days, respectively (Fig. 2b), indicating that *Ghd7.1* enhances *Ghd7*-driven *Hd1* function conversion like *Ghd8* in the ZS97 background.

Ghd7 alone does not promote *Hd1* function conversion in the MH63 background

Accordingly, four homozygous combinations of *Ghd7.1* and *Hd1* in the MH63 background were used to measure heading date under NLD. MH-G7g7.1H1 headed 11.3 days earlier than MH-G7g7.1h1, indicating that *Hd1* promoted heading in the MH63 background with *Ghd7* and *ghd7.1* (Fig. 2a). MH-G7G7.1H1 headed later than MH-G7G7.1h1 by 6.8 days (Fig. 2a), indicating that *Hd1* delayed heading in the MH63 background with *Ghd7.1*. Thus, *Ghd7.1* was also involved in *Hd1* function conversion in the MH63 background. Considering that *Ghd7* was functional in MH63 but nonfunctional in ZS97, it is possible that *Ghd7.1*-driven *Hd1* function conversion is dependent on *Ghd7*. Thus, MH-g7g7.1H1, MH-g7G7.1H1, MH-g7g7.1h1, and MH-g7G7.1h1 were constructed to eliminate the *Ghd7* effects. As expected, MH-g7g7.1H1 and MH-g7G7.1H1 headed 30.4 and 9.1 days earlier than MH-g7g7.1h1 and MH-g7G7.1h1, respectively (Fig. 2a), indicating that *Hd1* promoted heading. These results indicate that both *Ghd7* and *Ghd7.1* were required for *Hd1* function conversion in the MH63 background.

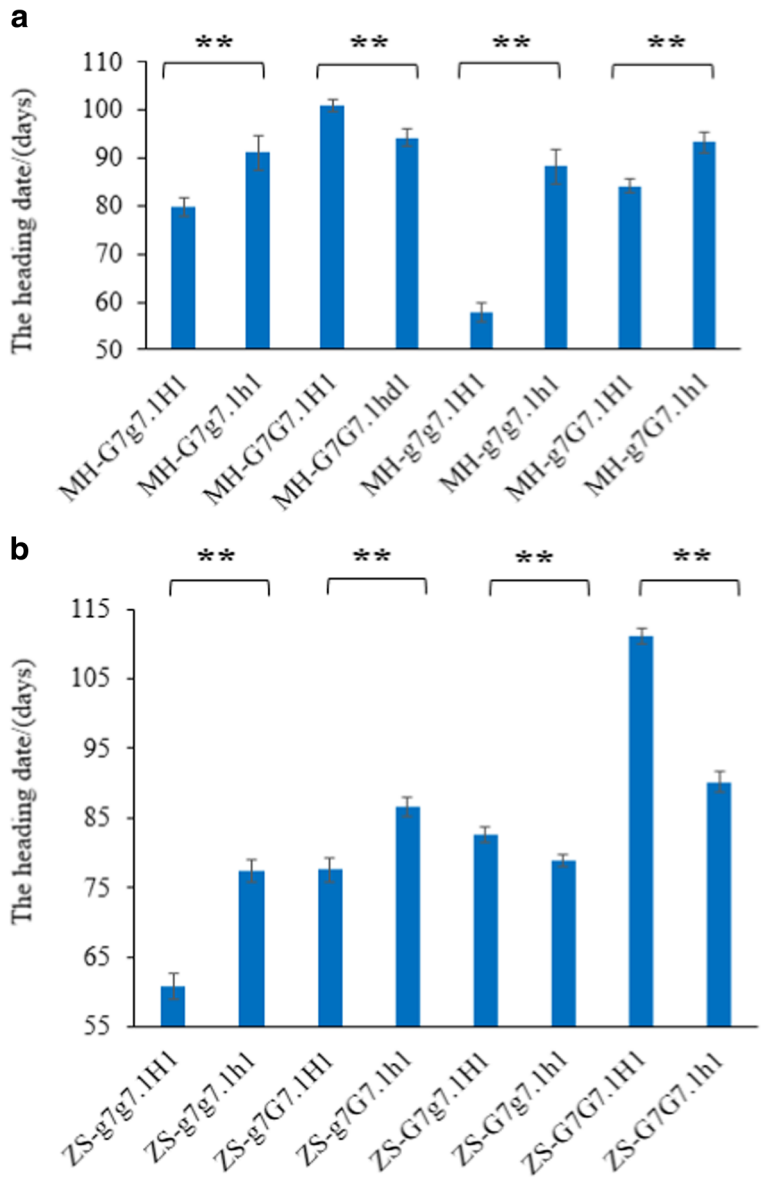

Fig. 2 The heading dates of *Ghd7*, *Ghd7.1*, and *Hd1* NILs in the ZS97 (**b**) and MH63 backgrounds (**a**) under NLD. **, significant difference in heading date at $P < 0.01$ by Student's *t* test

Photoperiod sensitivity of eight homozygous three-gene combinations of *Ghd7*, *Ghd7.1*, and *Hd1* in the MH63 and ZS97 backgrounds

We also measured heading dates for these eight homozygous combinations of *Ghd7*, *Ghd7.1*, and *Hd1* in the ZS97 and MH63 backgrounds under NLD, NSD, and SD. *Hd1* always promoted heading date on either the ZS97 or MH63 background under SD and NSD (Table 2).

PS was also estimated for the eight homozygous combinations of *Ghd7*, *Ghd7.1*, and *Hd1*. *Hd1* alone did not express photoperiod sensitivity in MH-g7g7.1H1 or ZS-g7g7.1H1 in which only *Hd1* was functional, whereas *Hd1* showed strong photoperiod sensitivity with the help of either *Ghd7* or *Ghd7.1* (Table 2). When both *Ghd7* and *Ghd7.1* were present, the combination of these three functional genes (ZS-G7G7.1H1 and MH-G7G7.1H1) possessed the strongest PS, at 50.6 d and 38.8 d (Table 2). When *Hd1* was nonfunctional, *ghd7Ghd7.1* and *Ghd7Ghd7.1* still possessed a strong PS of more than 20 days in the ZS97 background, while *Ghd7ghd7.1* and *ghd7ghd7.1* showed a weak PS of less than 8 days (Table 2).

Table 2 The photoperiod sensitivity of a series of three-gene combinations of *Ghd7*, *Ghd8*, and *Hd1* in the ZS97 and MH63 backgrounds

Genotype	HD (days)		PS	HD (days)
	LD	SD		NSD
ZS97 background				
ZS-G7G7.1H1	110.9 ± 1.3^{a}**	60.3 ± 0.4^{d}	50.6	107.8 ± 2.1^{e}
ZS-G7G7.1h1	89.3 ± 1.1^{b}**	64.8 ± 0.8^{b}	24.5	119.8 ± 1.9^{a}
ZS-g7G7.1H1	78.8 ± 1.3^{e}**	63.0 ± 0.3^{c}	15.8	111.3 ± 2.6^{d}
ZS-g7G7.1h1	86.4 ± 0.9^{c}**	63.8 ± 0.4^{bc}	22.6	118.2 ± 2.1^{ab}
ZS-G7g7.1H1	84.2 ± 1.0^{d}**	63.3 ± 0.4^{c}	20.9	109.8 ± 1.5^{d}
ZS-G7g7.1h1	79.4 ± 0.8^{e}**	73.8 ± 0.8^{a}	5.6	117.2 ± 0.9^{b}
ZS-g7g7.1H1	61.2 ± 2.1^{g}	60.0 ± 0.7^{d}	1.2	98.7 ± 4.8^{f}
ZS-g7g7.1h1	72.7 ± 1.5^{f}**	64.8 ± 0.4^{b}	7.9	113.5 ± 2.0^{c}
MH63 background				
MH-G7G7.1H1	106.3 ± 1.0^{a}**	67.5 ± 1.9^{c}	38.8	81.6 ± 3.6^{d}
MH-G7G7.1h1	95.3 ± 0.5^{b}**	84 ± 2.9^{b}	11.3	114.8 ± 3.2^{a}
MH-g7G7.1H1	87.3 ± 1.9^{c}**	67.6 ± 1.8^{c}	19.7	81.5 ± 2.4^{d}
MH-g7G7.1h1	93.5 ± 1.7^{b}**	88.3 ± 3.8^{a}	5.2	114.2 ± 4.4^{a}
MH-G7g7.1H1	79.5 ± 3.4^{e}**	59.0^{d}	20.5	80.3 ± 2.9^{d}
MH-G7g7.1h1	89.5 ± 1.9^{c}	89.4 ± 2.5^{a}	0.1	111.0 ± 2.7^{b}
MH-g7g7.1H1	55.5 ± 0.6^{f}	55.5 ± 0.6^{e}	0	78.5 ± 1.7^{e}
MH-g7g7.1h1	90 ± 3.5^{c}	89.6 ± 3.5^{a}	0.4	105.2 ± 5.0^{c}

HD the heading date, *LD* long day, *SD* short day, *NSD* natural short day, *PS* photoperiod sensitivity

There was no difference in other environmental factors except day length between LD and SD. Different letters within a column (a, b, c, d, e, and f) indicate a significance difference in multiple comparisons of the same genetic background at $P < 0.05$. **, significant difference in heading date between short-day conditions and long-day conditions at $P < 0.01$ by Student's *t* test

However, *Ghd7ghd7.1* and *ghd7ghd7.1* were insensitive to photoperiod in the MH63 background (Table 2).

Ghd8 joinly with *Ghd7.1* converts *Hd1* function in the ZS97 background

As mentioned above, *Ghd8* and *Ghd7.1* alone did not convert *Hd1* function, but *Ghd8* and *Ghd7.1* helped *Ghd7* greatly promoted *Hd1* function conversion in the ZS97 background (Figs. 1 and 2b). In addition, *Ghd7* alone or *Ghd7.1* alone did not convert *Hd1* function in the MH63 background, but when both were present, *Hd1* function conversion occurred (Fig. 2a). We hypothesized that *Ghd7.1* interacted with *Ghd8* to convert *Hd1* function without *Ghd7*. To verify this hypothesis, ZS-G7G8h1 was crossed with ZS-g7G7.1H1 to construct ZS-g7G8G7.1H1, ZS-g7G8G7.1h1, ZS-G7G8G7.1H1, and ZS-G7G8G7.1h1. As expected, ZS-g7G7.1G8H1 and ZS-G7G8G7.1H1 flowered 20.7 d and more than 35.8 d later than ZS-g7G8G7.1h1 and ZS-G7G8G7.1h1, respectively, under NLD (Fig. 3a and b). These findings suggest that *Ghd7.1* together with *Ghd8* converted *Hd1* without *Ghd7*.

Ehd1 and *Hd3a* are downregulated in the lines exhibiting *Hd1* function conversion under LD

In this study, *Hd1* (*g7g8g7.1H1*) promoted heading for approximately 11.3 d and 30.4 d in the ZS97 background and MH63 background, respectively (Figs. 1 and 2a), but *Hd1* function conversion was found in six genotypes. *Hd1* in the genotypes of ZS-G7G8G7.1H1 (Fig. 3b) and ZS-G7G8H1 (Fig. 1; Supplementary Table 2) extremely repressed flowering (for more than 35.8 d). *Hd1* strongly suppressed heading date (for about 21 d) in both ZS-G7G7.1H1 (Fig. 2b) and ZS-g7G8G7.1h1 (Fig. 3a). In the MH-G7G7.1H1 genotype, *Hd1* delayed

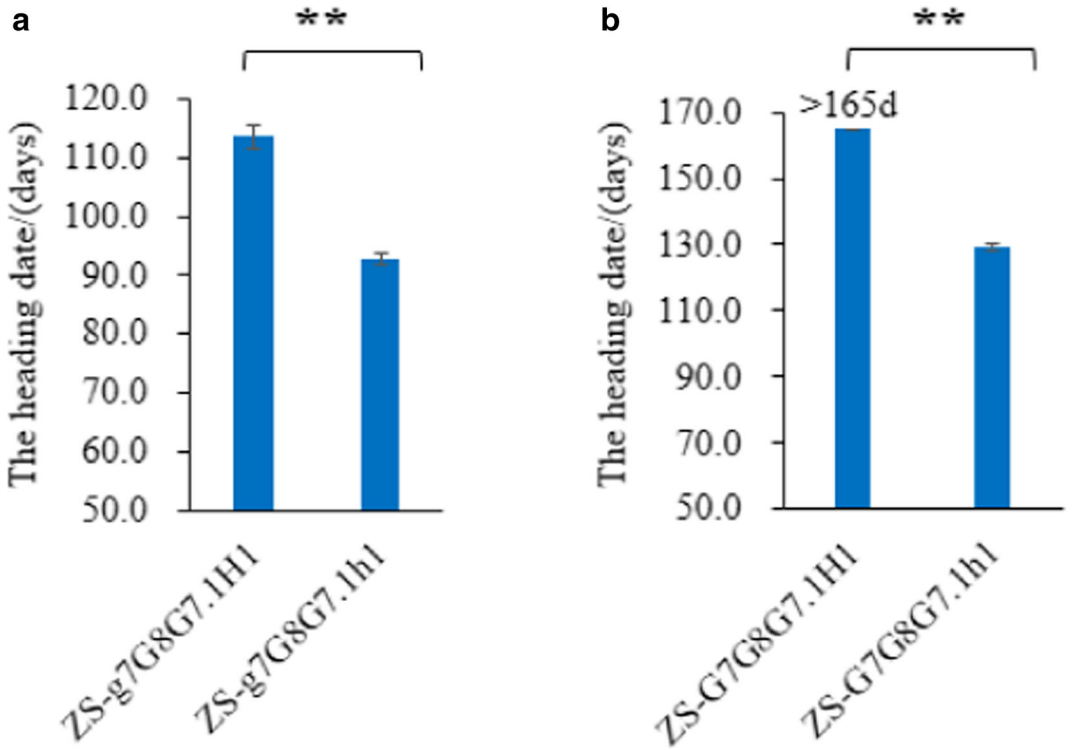

Fig. 3 *Ghd8* cooperates with *Ghd7.1* to convert *Hd1* function in the ZS97 background under NLD. **a** and **b**, the difference in heading date between *Hd1* and *hd1* in the ZS97 background with combinations of *ghd7Ghd8Ghd7.1* and *Ghd7Ghd8Ghd7.1*, respectively. ">165d", the heading date was more than 165 days, beyond our survey. **, significant difference in heading date at $P < 0.01$ by Student's *t* test

heading for 6.8 d (Fig. 2a). *Hd1* slightly delayed heading date in ZS-G7g8H1 (Fig. 1; Supplementary Table 2). To unveil the transcriptional mechanism of *Hd1* function conversion, we analyzed the expression levels of *Ehd1* and *Hd3a*, the main downstream targets of *Hd1*, in these six genotypes under LD. *Ehd1* and *Hd3a* expression levels in ZS-g7g8H1 and MH-g7g7.1H1 in which *Hd1* promoted heading were higher than ZS-g7g8h1 and MH-g7g7.1h1, respectively (Fig. 4a and b), but *Ehd1* and *Hd3a* expression levels in these six genotypes in which *Hd1* repressed flowering were much lower than in their corresponding *hd1* near-isogenic lines (Fig. 4a and b). In general, the more the flowering was delayed, the more the expression of *Hd3a* was inhibited (Fig. 4b). These results indicated that *Hd1* in the lines without *Hd1* function conversion upregulated *Ehd1* and *Hd3a* and promoted heading under LD, but *Hd1* downregulated *Ehd1* and *Hd3a* and suppressed heading in the lines with *Hd1* function conversion.

Discussion

Multiple gene combinations enhance *Hd1* function conversion

Hd1 was first reported to be sensitive to photoperiod because it promoted heading date under SD but delayed heading date under LD in rice (Yano et al. 2000). Then, *Hd1* was reported to constantly promote heading date in recent studies (Zhang et al. 2012; Du et al. 2017; Zhang et al. 2017). *Ghd7* and *Ghd8* are required to convert *Hd1* function from promoting to delaying heading under LD in the ZS97 and 93-11 backgrounds, respectively (Du et al. 2017; Zhang et al. 2017). The requirement of *Ghd7* for *Hd1* function conversion was confirmed in this study. However, *Ghd8* alone did not convert *Hd1* function (Fig. 1; Supplementary Table 2) but enhanced the effect of *Ghd7* on the *Hd1* function conversion in the ZS97 background (Fig. 1; Supplementary Table 2). Line 93-11 carries functional *Ghd7* and *Ghd8* (Zhang

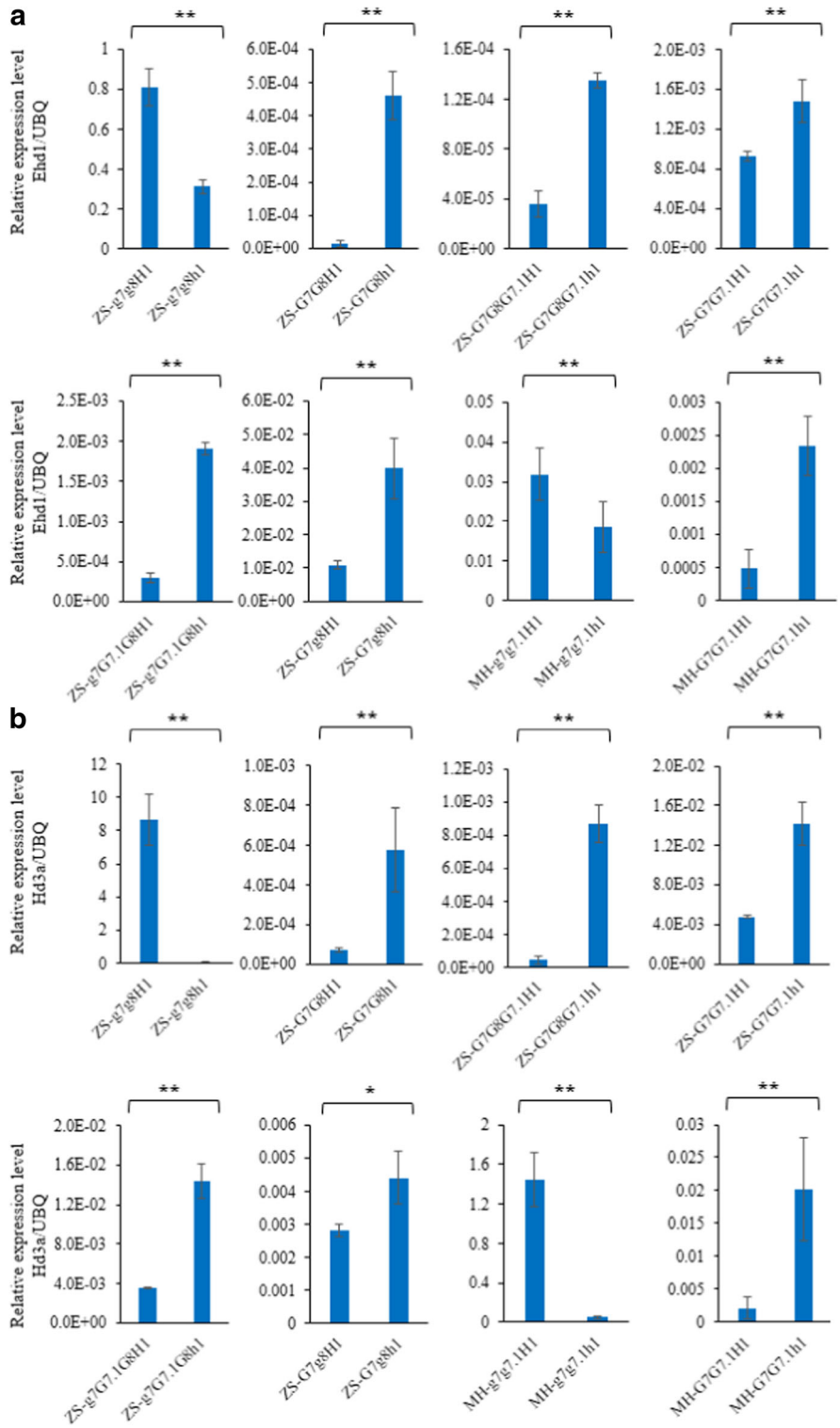

Fig. 4 The relative expression levels of *Ehd1* and *Hd3a* in *Hd1* function conversion lines. **a** and **b**, the relative expression levels of *Ehd1* and *Hd3a* in *Hd1* function conversion lines, respectively. Ubq was used as the internal reference. * and **, significant difference level at $P < 0.05$ and $P < 0.01$ by Student's *t* test, respectively

et al. 2015; Du et al. 2017). *Hd1* function conversion in 93-11 is caused by the interaction between *Ghd7* and *Ghd8* rather than *Ghd8* alone. We uncovered that *Ghd7.1* alone did not drive *Hd1* conversion, but it enhanced *Ghd7* to promote *Hd1* function conversion (Fig. 2a and b). Moreover, *Ghd7.1* together with *Ghd8* promoted *Hd1* function conversion without *Ghd7* (Fig. 3a). MH-G7g7.1H1 headed earlier than MH-G7g7.1h1 (Fig. 2a), which was different from that in the ZS97 background (Figs. 1 and 2b). Other genes probably participate in the regulation of *Ghd7*-driven *Hd1* function conversion, which was absent in MH63 but present in ZS97. Taken together, these findings show that *Hd1* function conversion was driven by multiple gene combinations among *Ghd7*, *Ghd8*, *Ghd7.1*, and other unknown factors rather than a single gene.

The NF-Y-CCT complexes possibly underlie the mechanism of *Hd1* function conversion in rice

Ghd7, *Ghd7.1*, and *Hd1* all encode CCT-domain-containing proteins, while *Ghd8* encodes an NF-YB protein (Yano et al. 2000; Xue et al. 2008; Yan et al. 2011; Hori et al. 2013). NF-YB family proteins form a heterotrimer complex with NF-YC and NF-YA family proteins in a wide variety of eukaryotes (Petroni et al. 2012). *CO*, an ortholog of *Hd1* in Arabidopsis, containing a CCT domain that is highly conserved with the NF-YA binding domain, interacts with NF-YB and NF-YC family proteins physically to form an NF-Y-CO complex through its CCT domain (Wenkel et al. 2006; Hou et al. 2014). In rice, the Ghd8-Hd1, Ghd7-Hd1, and OsNF-YA-Ghd8-OsNF-YC complexes have been identified in vitro or in vivo. Hd1 interacts with OsNF-YA, OsNF-YB, and OsNF-YC in yeast, and Ghd7.1 interacts with Ghd8 in yeast (Kim et al. 2016; Nemoto et al. 2016; Du et al. 2017; Goretti et al. 2017; Zhang et al. 2017; Zhu et al. 2017). These findings suggest that the NF-YC-CCT complexes, such as Ghd7-Ghd8-Hd1-OsNF-YC, Ghd7-OsNF-YB-Hd1-OsNF-YC, Ghd7.1-Ghd8-Hd1-OsNF-YC, and Ghd7-Ghd8-Ghd7.1-OsNF-YC, might be formed in rice. Identification of these complexes would be helpful in addressing the mechanism of *Hd1* function conversion mediated by *Ghd7*, *Ghd8*, and *Ghd7.1*.

The Ghd8-Hd1 complex increases the H3K27me3 level on the Ghd8-binding site in the *Hd3a* promoter and leads to inhibited *Hd3a* expression (Du et al. 2017). In Arabidopsis, NF-Y-CO regulates flowering time by altering the histone H3K27me3 level of the *FT* and *SOC1* promoter (Hou et al. 2014; Liu et al. 2018). Thus, we assume that NF-Y-CCT complexes in rice are necessary to determine *Hd1* function conversion by upregulating the H3K27me3 level of *Ehd1* and *Hd3a* and covering up the effect of transcriptional activation of Hd1.

Developing varieties for global and local ecological areas according to photoperiod sensitivity of multiple gene combinations

Photoperiod sensitivity is crucial for rice adaptation to cropping areas, and photoperiod insensitivity is a prerequisite for cropping elite varieties in wide regions. A positive correlation was observed between heading date and grain yield (Hu et al. 2019). *Ghd7*, *Ghd7.1*, *Ghd8*, and *Hd1* are sensitive to photoperiod. Nonfunctional alleles of these photoperiod-sensitive genes show photoperiod insensitivity, which leads to similar heading dates either in LD or in SD (Yano et al. 2000; Xue et al. 2008; Yan et al. 2011; Hori et al. 2013). When these functional and nonfunctional alleles combined with each other, they had varied PS. The four gene combinations of *Ghd7Ghd7.1Hd1*, *Ghd7Ghd7.1hd1*, *ghd7Ghd7.1Hd1*, and *Ghd7ghd7.1Hd1* possessed strong PS. Cultivars carrying these gene combinations should have long life cycles, even under SD, and adapt to subtropical regions. The three combinations *Ghd7ghd7.1hd1*, *ghd7ghd7.1Hd1*, and *ghd7ghd7.1hd1* were weakly sensitive or insensitive to photoperiod. They had similar heading dates between LD and SD. Of them, the combination of *Ghd7ghd7.1hd1* had 80 and 90 days to heading under conditions in the ZS97 and MH63 backgrounds, respectively, which makes them promising to plant in different regions for high yield production. The combination of *ghd7ghd7.1Hd1* had very short heading times of 55 and 60 days in the ZS97 and MH63 backgrounds, respectively. They are adapted to the northeast China cropping regions or as early rice in central China, where day length is long day in the rice growing season. Above all, estimation of the PS index would help breeders to develop photoperiod-insensitive varieties that could be delivered to widespread ecological areas.

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Author's contribution ZYZ and BZ are equal contributors. ZYZ and BZ performed the construction of NILs, data collection and analysis, field experiments, and gene expression detection. FXQ and HW contributed to QTLs genotyping. ZXL and YZX designed the research. ZYZ, BZ, and YZX wrote and revised the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

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