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## Quantitative trait loci for large sink capacity enhance rice grain yield under free-air CO<sub>2</sub> enrichment conditions

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The global atmospheric CO<sub>2</sub> concentration has been increasing annually. To determine the trait that effectively increases rice (*Oryza sativa* L.) grain yield under increased atmospheric CO<sub>2</sub> concentrations, as predicted in the near future, we grew a chromosome segment substitution line (CSSL) and a near-isogenic line (NIL) producing high spikelet numbers per panicle (CSSL-*GN1* and NIL-*APO1*, respectively) under free-air CO<sub>2</sub> enrichment (FACE) conditions and examined the effects of a large sink capacity on grain yield, its components, and growth-related traits under increased atmospheric CO<sub>2</sub> concentrations. Under ambient conditions, CSSL-*GN1* and NIL-*APO1* exhibited a similar grain yield to Koshihikari, as a result of the trade-off between increased spikelet number and reduced grain filling. However, under FACE conditions, CSSL-*GN1* and NIL-*APO1* had an equal or a higher grain yield than Koshihikari because of the higher number of spikelets and lower reduction in grain filling. Thus, the improvement of source activity by increased atmospheric CO<sub>2</sub> concentrations can lead to enhanced grain yield in rice lines that have a large sink capacity. Therefore, introducing alleles that increase sink capacity into conventional varieties represents a strategy that can be used to develop high-yielding varieties under increased atmospheric CO<sub>2</sub> concentrations, such as those predicted in the near future.

Crop growth and yield are affected by global changes in the environment, such as increasing atmospheric carbon dioxide (CO<sub>2</sub>) concentrations and air temperature<sup>1</sup>. The atmospheric CO<sub>2</sub> concentration has increased steadily from 280 μmol mol<sup>-1</sup> before the Industrial Revolution, to 400 μmol mol<sup>-1</sup> in 2015<sup>2</sup>, and is projected to continue to increase over the course of this century.

The world's population is estimated to reach 9.1 billion people by 2050<sup>3</sup>. To feed such a large number of people, global food production must be increased substantially. Rice (*Oryza sativa* L.) is eaten by nearly half of the world's population and is a staple food for most population<sup>4</sup>. Because arable land for rice is limited, improving rice yield per unit area is essential to resolve global food issues. However, the increased rice yield per unit area has been reduced in recent years throughout the world<sup>5</sup>.

Terrestrial plants containing a C<sub>3</sub> photosynthetic pathway, including rice, are positively influenced by increased atmospheric CO<sub>2</sub> through photosynthetic rates and water-use efficiency<sup>6,7</sup>. To produce high grain yield in rice, a large sink capacity is needed, as well as the ability to produce high levels of carbohydrates and to translocate them to the sink. Rice grain yield is thought to be improved in response to rising atmospheric CO<sub>2</sub>

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concentrations through an increased number of spikelets per square meter<sup>8–12</sup>. High-yielding varieties with a large sink capacity (spikelet number per square meter  $\times$  single grain weight) have a higher grain-yield response to increased CO<sub>2</sub> concentrations than conventional varieties with a general sink capacity<sup>11</sup>. Furthermore, there is a positive correlation between grain-yield response to increased CO<sub>2</sub> concentrations and sink capacity. However, such high-yielding varieties with a large sink capacity generally possess other traits that allow them to produce a high grain yield. Therefore, direct evidence regarding the relationship between grain-yield response and increased CO<sub>2</sub> concentration, and sink capacity is lacking.

Recent progress in rice genomics has facilitated genetic analyses of quantitative traits such as grain yield, and some genes regulating spikelet number per panicle of rice have been identified<sup>13,14</sup>. One quantitative trait loci (QTL) *GN1a*, results in the production of more spikelets per panicle in the presence of an allele from an *indica* high-yielding variety than with that from a *japonica* conventional variety<sup>13</sup>. *GN1a* encodes the enzyme cytokinin oxidase/dehydrogenase (OsCKX2). In addition, *GN1b*, which is estimated to lie in the vicinity of *GN1a*, may also increase spikelet number per panicle with the allele from an *indica* high-yielding variety. Similarly, an allele of *APO1* derived from an *indica* high-yielding variety was found to produce a higher spikelet number per panicle than that from a *japonica* conventional variety<sup>14</sup>. However, in some field experiments performed under ambient conditions, near-isogenic lines (NIL) or chromosome segment substitution lines (CSSL) possessing such favorable alleles in the *japonica* genetic background did not have a higher grain yield due to the lower percentage of filled spikelets and 1000-grain weight<sup>15,16</sup>, suggesting a lack of source activity relative to their large sink capacity.

To date, several laboratory and chamber experiments investigating the plant growth response to increased atmospheric CO<sub>2</sub> concentrations have been conducted and reported<sup>17</sup>. However, plant growth responses in the laboratory or under chamber conditions may differ from those observed under field conditions. Free-air CO<sub>2</sub> enrichment (FACE) experiments represent a promising method to grow plants at controlled levels of elevated CO<sub>2</sub> under fully open-air field conditions<sup>7</sup> in order to investigate yield response to CO<sub>2</sub> increases.

Increased atmospheric CO<sub>2</sub> concentrations lead to an increase in the dry matter yield of rice<sup>10,11</sup>, indicating that source activity is raised. A previous rice FACE study showed that a variety Takanari with a large sink capacity is higher yielding and more responsive to increased CO<sub>2</sub> concentrations than a conventional cultivar Koshihikari<sup>11</sup>. To raise yield potentials under increased CO<sub>2</sub> concentrations efficiently and effectively, we need to understand the effects of QTLs that can enhance sink capacity on yield performance in increased CO<sub>2</sub> under open field conditions. Recently, a CSSL and a NIL were developed carrying *GN1* and *APO1* alleles, respectively, from Takanari in the Koshihikari genetic background<sup>16</sup>. We therefore hypothesized that *GN1* and *APO1* alleles, which produce a higher spikelet number per panicle, have a high grain yield as a result of enhanced source activity under increased atmospheric CO<sub>2</sub> concentrations. To test this hypothesis, we grew a conventional variety, Koshihikari, and CSSL-*GN1* and NIL-*APO1* with the Koshihikari genetic background under FACE conditions and examined the effects of a large sink capacity on grain yield, its components, and growth related traits under increased atmospheric CO<sub>2</sub> concentrations (200  $\mu\text{mol mol}^{-1}$  above the ambient CO<sub>2</sub>). On the basis of the results, we consider whether introducing such alleles to conventional varieties represents an effective method of increasing grain yield under increased atmospheric CO<sub>2</sub> concentrations, such as those predicted in the near future.

## Results

There were no significant interactions between year and CO<sub>2</sub> concentration or among year, CO<sub>2</sub> concentration, and genotype for grain yield, so data were combined over two years.

**Grain yield.** Grain yield was affected by CO<sub>2</sub> concentration and genotype. There was an interaction between CO<sub>2</sub> concentration and genotype for grain yield (Table 1). In Koshihikari, grain yield did not differ between CO<sub>2</sub> concentrations (FACE/ambient = 1.08). However, in CSSL-*GN1* and NIL-*APO1*, grain yield under FACE conditions was increased compared with that under ambient conditions (FACE/ambient = 1.21 and 1.19, respectively). Under ambient conditions, CSSL-*GN1* and NIL-*APO1* had almost the same grain yield as Koshihikari. In contrast, under FACE conditions, CSSL-*GN1* and NIL-*APO1* had an equal or a higher grain yield than Koshihikari.

**Grain-yield components and harvest index.** Grain-yield components relating to spikelet number were affected by CO<sub>2</sub> concentration and genotype (Table 1). In all tested genotypes, spikelet number per square meter, panicle number per square meter, and spikelet number per panicle did not differ between CO<sub>2</sub> concentrations. Under both CO<sub>2</sub> concentrations, CSSL-*GN1* and NIL-*APO1* had a lower panicle number per square meter than Koshihikari, but had a much higher spikelet number per panicle. Consequently, CSSL-*GN1* and NIL-*APO1* had a higher spikelet number per square meter than Koshihikari.

Grain yield components relating to grain filling were influenced by CO<sub>2</sub> concentration and genotype (Table 1). In all tested genotypes, the percentage of filled spikelets under FACE conditions was increased compared with that under ambient conditions. Under both CO<sub>2</sub> concentrations, CSSL-*GN1* had low percentage of filled spikelets and 1000-grain weight relative to Koshihikari, whereas NIL-*APO1* had almost the same percentage and weight as Koshihikari.

There was an interaction between CO<sub>2</sub> concentration and genotype for harvest index. Under ambient condition, CSSL-*GN1* had the lowest harvest index in all tested genotypes. However, under FACE condition, CSSL-*GN1* had a similar harvest index to Koshihikari. On the other hand, NIL-*APO1* had the highest harvest index in all tested genotypes consistently across two CO<sub>2</sub> concentrations.

**Growth-related traits at heading.** The effects of CO<sub>2</sub> concentration and genotype on growth-related traits at heading were examined (Table 2). In all tested genotypes, DM weight, stem DM weight, and NSC concentration under FACE conditions were increased compared with those under ambient conditions. As a result, the NSC

CO <sub>2</sub> concentration	Genotype	Grain Yield (g m <sup>-2</sup> )	Spikelet number (× 10 <sup>3</sup> m <sup>-2</sup> )	Panicle number (m <sup>-2</sup> )	Spikelet number (panicle <sup>-1</sup> )	Percentage of filled spikelets (%)	1000-grain weight (g)	Harvest Index
CO <sub>2</sub> concentration (C)								
FACE		813	47.0	333	143	85.4	20.5	0.41
Ambient		702	44.4	326	138	77.6	20.6	0.40
Genotype (G)								
	Koshihikari	735b <sup>†</sup>	40.5c	358a	113c	86.5a	21.0a	0.39b
	CSSL-GNI	724b	51.3a	322b	160a	72.9b	19.4b	0.39b
	NIL-APOI	814a	45.3b	308b	147b	85.0a	21.1a	0.43a
C × G								
FACE	Koshihikari	763b	40.6	358	114	89.2	21.1	0.40b
	CSSL-GNI	791abA <sup>‡</sup>	53.4	328	163	78.0	19.1	0.40bA
	NIL-APOI	886aA	47.0	313	151	89.0	21.2	0.43a
Ambient	Koshihikari	708ab	40.3	359	113	83.9	21.0	0.39b
	CSSL-GNI	656bB	49.3	316	157	67.7	19.7	0.38cB
	NIL-APOI	743aB	43.5	304	144	81.0	21.1	0.42a
ANOVA								
CO <sub>2</sub> concentration (C)		NS <sup>§</sup>	NS	NS	NS	**	NS	§
Genotype (G)		**	**	**	**	**	**	**
C × G		*	NS	NS	NS	NS	**	§

**Table 1.** Mean grain yield, its components, and harvest index as affected by different CO<sub>2</sub> concentrations and genotypes averaged for two years (2012 and 2013). \*Significant at P < 0.05. \*\*Significant at P < 0.01. <sup>†</sup>Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05). <sup>‡</sup>Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) among genotypes for a given CO<sub>2</sub> concentration. Means within a column followed by the same uppercase letter do not differ significantly (P < 0.05) between CO<sub>2</sub> concentrations for a given genotype. <sup>§</sup>Significant at P < 0.10. <sup>¶</sup>Not significant at P < 0.10.

CO <sub>2</sub> concentration	Genotype	DM weight (g m <sup>-2</sup> )	Stem DM weight (g m <sup>-2</sup> )	NSC concentration (g kg <sup>-1</sup> )	NSC amount (g m <sup>-2</sup> )	LAI
CO <sub>2</sub> concentration (C)						
FACE		1152	713	370	264	4.33
Ambient		1038	609	332	203	4.67
Genotype (G)						
	Koshihikari	1069	674	366	248	4.37
	CSSL-GNI	1093	643	335	217	4.60
	NIL-APOI	1124	666	351	235	4.53
C × G						
FACE	Koshihikari	1127	731	380	277	4.12
	CSSL-GNI	1151	694	359	250	4.48
	NIL-APOI	1179	714	371	265	4.40
Ambient	Koshihikari	1011	617	353	219	4.62
	CSSL-GNI	1036	591	312	185	4.73
	NIL-APOI	1069	619	332	205	4.66
ANOVA						
CO <sub>2</sub> concentration (C)		*	**	*	**	NS
Genotype (G)		NS <sup>†</sup>	NS	NS	NS	NS
C × G		NS	NS	NS	NS	NS

**Table 2.** Mean dry matter (DM) weight, nonstructural carbohydrate (NSC) concentration and its amount in the leaf sheaths plus stems, and leaf area index (LAI) at heading as affected by different CO<sub>2</sub> concentrations and genotypes averaged for two years (2012 and 2013). \*Significant at P < 0.05. \*\*Significant at P < 0.01. <sup>†</sup>Not significant at P < 0.10.

amount under FACE conditions was larger than that under ambient conditions. In addition, LAI did not differ between CO<sub>2</sub> concentrations and among genotypes.

**Growth-related traits at maturity.** The effects of CO<sub>2</sub> concentration and genotype on growth-related traits at maturity were examined (Table 3). In all tested genotypes, DM weight, stem DM weight, and NSC

CO <sub>2</sub> concentration	Genotype	DM weight (g m <sup>-2</sup> )	Stem DM weight (g m <sup>-2</sup> )	NSC concentration (g kg <sup>-1</sup> )	NSC amount (g m <sup>-2</sup> )	ΔW (g m <sup>-2</sup> )
CO <sub>2</sub> concentration (C)						
FACE		1991	786	305	241	833
Ambient		1777	664	264	176	739
Genotype (G)						
	Koshihikari	1874	762a <sup>†</sup>	294	227a	805
	CSSL- <i>GNI</i>	1864	691b	286	199b	771
	NIL- <i>APO1</i>	1913	722ab	273	200b	790
C × G						
FACE	Koshihikari	1938	830	326	271aA <sup>‡</sup>	811
	CSSL- <i>GNI</i>	1982	738	296	219bA	831
	NIL- <i>APO1</i>	2051	789	294	233bA	872
Ambient	Koshihikari	1811	693	262	183B	800
	CSSL- <i>GNI</i>	1746	644	276	179B	711
	NIL- <i>APO1</i>	1775	654	252	166B	707
ANOVA						
CO <sub>2</sub> concentration (C)		§	*	**	**	NS
Genotype (G)		NS <sup>§</sup>	**	NS	*	NS
C × G		NS	NS	NS	§	NS

**Table 3.** Mean dry matter (DM) weight, nonstructural carbohydrate (NSC) concentration, and its amount in the leaf sheaths plus stems at maturity, and DM increase from heading to maturity (ΔW) affected by different CO<sub>2</sub> concentrations and genotypes averaged for two years (2012 and 2013). \*Significant at  $P < 0.05$ . \*\*Significant at  $P < 0.01$ . <sup>†</sup>Means within a column followed by the same lowercase letter do not differ significantly ( $P < 0.05$ ). <sup>‡</sup>Means within a column followed by the same lowercase letter do not differ significantly ( $P < 0.05$ ) among genotypes for a given CO<sub>2</sub> concentration. Means within a column followed by the same uppercase letter do not differ significantly ( $P < 0.05$ ) between CO<sub>2</sub> concentrations for a given genotype. <sup>§</sup>Significant at  $P < 0.10$ . <sup>¶</sup>Not significant at  $P < 0.10$ .

	Spikelet number (× 10 <sup>3</sup> m <sup>-2</sup> )	Percentage of filled spikelets (%)	1000-grain weight (g)	NSC amount at heading (g m <sup>-2</sup> )	ΔW <sup>†</sup> (g m <sup>-2</sup> )
Grain yield	-0.046	0.925***	0.429	0.874***	0.610 <sup>‡</sup>
Percentage of filled spikelets	-0.375	—	0.611 <sup>‡</sup>	0.762**	0.574

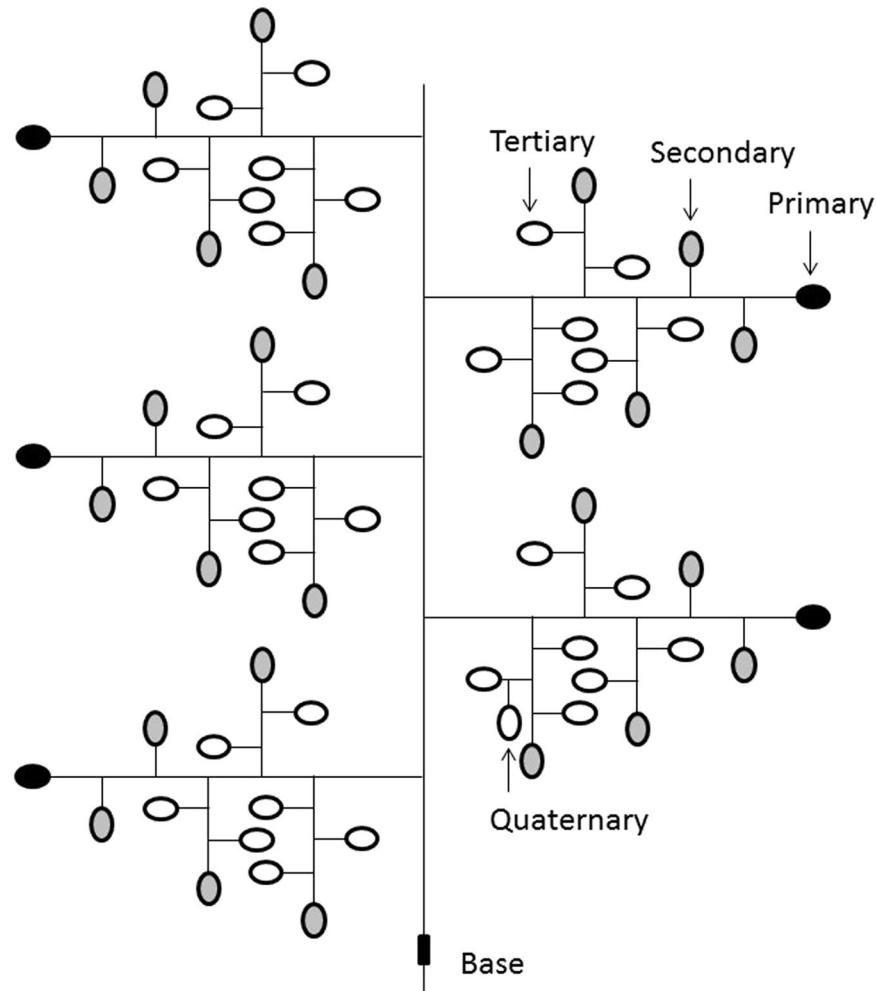
**Table 4.** Pearson correlation ( $r$ ) analysis for agronomic traits in CSSL-*GNI* and NIL-*APO1* in 2012 and 2013. \*\*\*Significant at  $P < 0.001$ . \*\*Significant at  $P < 0.01$ . <sup>†</sup>Dry matter increase from heading to maturity. <sup>‡</sup>Significant at  $P < 0.10$ .

concentration under FACE conditions were increased compared with those under ambient conditions. There was an interaction between CO<sub>2</sub> concentration and genotype for the NSC amount. In all tested genotypes, NSC amount was increased compared with that under ambient condition. Under ambient condition, the NSC amount did not differ among genotypes. However, under FACE condition, CSSL-*GNI* and NIL-*APO1* had a lower NSC amount than Koshihikari. In addition, ΔW did not differ between CO<sub>2</sub> concentrations and among genotypes.

**Relationships between agronomic traits in CSSL-*GNI* and NIL-*APO1*.** Grain yield of CSSL-*GNI* and NIL-*APO1* was positively correlated with the percentage of filled spikelets, NSC amount at heading, and ΔW grain yield, but not with spikelet number per square meter and 1000-grain weight (Table 4). Also, the percentage of filled spikelets was positively correlated with correlated with 1000-grain weight and NSC amount at heading, but not with spikelet number per square meter and ΔW.

**Percentage of spikelet and spikelet weight at each position in the panicle.** The percentage of spikelets at each position in the panicle was affected by genotype but not by CO<sub>2</sub> concentration (Fig. 1 and Table 5). Under both CO<sub>2</sub> concentrations, CSSL-*GNI* and NIL-*APO1* had a lower percentage of primary and secondary spikelets than Koshihikari, but a higher percentage of tertiary spikelets.

Spikelet weight at each position in the panicle was influenced by CO<sub>2</sub> concentration and genotype (Fig. 1 and Table 5). There was an interaction between CO<sub>2</sub> concentration and genotype for primary spikelet weight. In Koshihikari, the primary spikelet weight under FACE condition was increased compared with that under ambient condition. However, in CSSL-*GNI* and NIL-*APO1*, the primary spikelet weight did not differ between CO<sub>2</sub> concentrations. Under ambient conditions, primary spikelet weight did not differ among genotypes. However, under FACE conditions, CSSL-*GNI* had a low primary spikelet weight relative to Koshihikari, whereas NIL-*APO1* had



**Figure 1.** Panicle structure of rice.

a similar primary spikelet weight to Koshihikari. In all tested genotypes, the tertiary spikelet weight under FACE conditions was increased compared with that in ambient. Under both CO<sub>2</sub> concentrations, CSSL-*GNI* had low secondary and tertiary spikelet weights relative to Koshihikari, whereas NIL-*APO1* had almost the same secondary and tertiary spikelet weights as Koshihikari.

**Spikelet number and weight per square meter at each position in the panicle.** Spikelet number at each position in the panicle per square meter were affected by CO<sub>2</sub> concentration and genotype (Fig. 1 and Table 6). In all tested genotypes, the tertiary spikelet number per square meter under FACE conditions was increased compared with that under ambient conditions. Under both CO<sub>2</sub> concentrations, CSSL-*GNI* had a higher secondary spikelet number than Koshihikari, whereas NIL-*APO1* had almost the same number as Koshihikari. Similarly, CSSL-*GNI* had the highest tertiary spikelet number, followed by NIL-*APO1* and Koshihikari.

Spikelet weight at each position in the panicle per square meter was influenced by CO<sub>2</sub> concentration, but not by genotype (Fig. 1 and Table 6). In all genotypes, tertiary spikelet weight under FACE conditions was increased compared with that under ambient conditions.

## Discussion

The results of previous studies have indicated that CSSL and NIL, carrying *GNI* or *APO1* alleles derived from high-yielding *indica* varieties, did not exhibit high grain yield despite their large sink capacity<sup>15,16</sup>. In the present study, CSSL-*GNI* and NIL-*APO1* had almost the same grain yield as Koshihikari under ambient conditions because of the trade-off between increased spikelet number and reduced grain filling (Table 1). Furthermore, CSSL-*GNI* had a lower grain yield than NIL-*APO1*. The low grain yield of CSSL-*GNI* may result from its extremely large sink capacity. These results confirmed that high source activity is required to increase grain yield in rice lines that have a large sink capacity.

In contrast to this, under FACE conditions, CSSL-*GNI* and NIL-*APO1* had an equal or a higher grain yield than Koshihikari because of the higher number of spikelets and lower reduction in grain filling. In addition,

CO <sub>2</sub> concentration	Genotype	Percentage of spikelet				Spikelet weight			
		Primary (%)	Secondary (%)	Tertiary (%)	Quaternary (%)	Primary (mg spikelet <sup>-1</sup> )	Secondary (mg spikelet <sup>-1</sup> )	Tertiary (mg spikelet <sup>-1</sup> )	Quaternary (mg spikelet <sup>-1</sup> )
CO <sub>2</sub> concentration (C)									
FACE		6.7	52.8	40.4	0.0	24.6	23.6	18.7	0.3
Ambient		6.9	53.6	39.6	0.0	23.9	23.3	15.5	0.0
Genotype (G)									
	Koshihikari	7.7a <sup>†</sup>	57.5a	34.8b	0.0	24.7a	24.5a	19.2a	0.0
	CSSL-GNI	6.2b	50.5b	43.3a	0.0	23.6b	21.9b	14.3b	0.0
	NIL-APO1	6.6b	51.5b	41.9a	0.1	24.5a	24.0a	17.8a	0.5
C × G									
FACE	Koshihikari	7.7	57.6	34.7	0.0	25.6aA <sup>‡</sup>	25.1	21.5	0.0
	CSSL-GNI	6.0	49.8	44.2	0.0	23.3b	21.8	15.1	0.0
	NIL-APO1	6.5	51.0	42.5	0.1	24.8a	24.0	19.4	1.0
Ambient	Koshihikari	7.7	57.4	34.8	0.0	23.8B	23.9	16.9	0.0
	CSSL-GNI	6.3	51.3	42.5	0.0	23.9	22.1	13.4	0.0
	NIL-APO1	6.7	52.1	41.3	0.0	24.1	24.1	16.1	0.0
ANOVA									
CO <sub>2</sub> concentration (C)		NS <sup>‡</sup>	NS	NS	NS	NS	NS	*	NS
Genotype (G)		**	**	**	NS	§ <sup>§</sup>	*	*	NS
C × G		NS	NS	NS	NS	§	NS	NS	NS

**Table 5.** Mean percentage of spikelet and spikelet weight per spikelet at each position (primary, secondary, tertiary, and quaternary) in the panicles as affected by different CO<sub>2</sub> concentrations and genotype averaged for two years (2012 and 2013). \*Significant at P < 0.05. \*\*Significant at P < 0.01. <sup>†</sup>Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05). <sup>‡</sup>Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05) among genotypes for a given CO<sub>2</sub> concentration. Means within a column followed by the same uppercase letter do not differ significantly (P < 0.05) between CO<sub>2</sub> concentrations for a given genotype. <sup>§</sup>Significant at P < 0.10. <sup>¶</sup>Not significant at P < 0.10.

although the effect of increased atmospheric CO<sub>2</sub> concentration on spikelet number was not clear in the present study (Table 1), spikelet number per square meter was reportedly increased by increased atmospheric CO<sub>2</sub> concentrations<sup>8–12, 18</sup>.

Grain carbohydrates are derived from both accumulated carbohydrates (i.e., NSC) in the leaf sheaths plus stems at heading and photosynthetic products during grain filling (i.e.,  $\Delta W$ )<sup>19</sup>. The contributions of NSC at heading and  $\Delta W$  to grain carbohydrates range from 0 to 40% and 60 to 100%, respectively, under most weather conditions during grain filling. However, NSC at heading is considered to be important to stabilize grain yield under unfavorable weather conditions during grain filling, because it compensates for the reduction in  $\Delta W$ <sup>20–22</sup>. The NSC amount at heading in the present study were increased by increased atmospheric CO<sub>2</sub> concentrations in all tested genotypes (Tables 2). The large amount of NSC at heading resulted from the stem DM weight, and NSC concentration at heading. The results of a previous study indicated that grain yield was closely related to crop growth rate (CGR) during the late reproductive period<sup>23</sup>. The high CGR led to the production of a high number of spikelets per square meter and a high level of NSC amount at heading, which was associated with the rapid translocation of NSC to the panicle during the initial period of grain filling. In the present study, the grain yield of CSSL-GNI and NIL-APO1 was related to the percentage of filled spikelets and NSC amount at heading, and the percentage of filled spikelets was related to the NSC amount at heading (Table 4). In addition, the results of a recent study revealed that spikelet sterility of varieties with a high number of spikelets was caused by a lack of assimilate supply around flowering<sup>24</sup>. Thus, improved source activity in response to increased atmospheric CO<sub>2</sub> concentration can enhance grain yield in rice lines that have a large sink capacity.

In the present study, in all tested genotypes, NSC amount at maturity was increased by increased atmospheric CO<sub>2</sub> concentrations (Table 3), whereas harvest index was not decreased by atmospheric CO<sub>2</sub> concentrations in CSSL-GNI (Table 1). Under ambient condition, CSSL-GNI had the lowest harvest index in all tested genotypes. However, under FACE condition, CSSL-GNI had a similar harvest index to Koshihikari. Varieties with a small sink capacity have a large amount of NSC at the late grain filling stage<sup>25, 26</sup>. Hence, under FACE conditions, Koshihikari may not be able to translocate a large amount of carbohydrates to the panicle due to its limited sink capacity, whereas CSSL-GNI and NIL-APO1 are able to this due to their large sink capacity.

Primary spikelets have an advantage for grain filling over secondary spikelets<sup>27</sup>, which is advantageous for grain filling compared with tertiary and quaternary spikelets. In all tested genotypes, the number of tertiary spikelets per square meter in the present study was increased by increased atmospheric CO<sub>2</sub> concentrations (Fig. 1 and Table 6). Under both CO<sub>2</sub> concentrations, CSSL-GNI and NIL-APO1 had a higher percentage of tertiary spikelets and more tertiary spikelets per square meter than Koshihikari (Tables 5 and 6), indicating that CSSL-GNI and NIL-APO1 possess more disadvantaged spikelets for grain filling under FACE conditions. However, in CSSL-GNI and NIL-APO1 as well as in Koshihikari, the tertiary spikelet weight per spikelet and the spikelet weight per square meter was increased by increased atmospheric CO<sub>2</sub> concentration. Therefore, in CSSL-GNI

CO <sub>2</sub> concentration	Genotype	Spikelet number				Spikelet weight			
		Primary (×10 <sup>3</sup> m <sup>-2</sup> )	Secondary (×10 <sup>3</sup> m <sup>-2</sup> )	Tertiary (×10 <sup>3</sup> m <sup>-2</sup> )	Quaternary (×10 <sup>3</sup> m <sup>-2</sup> )	Primary (g m <sup>-2</sup> )	Secondary (g m <sup>-2</sup> )	Tertiary (g m <sup>-2</sup> )	Quaternary (g m <sup>-2</sup> )
CO <sub>2</sub> concentration (C)									
FACE		3.1	24.6	19.2	0.0	77	579	350	0.0
Ambient		3.0	23.7	17.7	0.0	72	552	271	0.0
Genotype (G)									
	Koshihikari	3.1	23.3b <sup>†</sup>	14.1c	0.0	77	569	271	0.0
	CSSL-GNI	3.2	26.0a	22.2a	0.0	74	570	319	0.0
	NIL-APOI	3.0	23.2b	19.0b	0.0	72	558	341	0.0
C × G									
FACE	Koshihikari	3.1	23.4	14.2	0.0	80	584	305	0.0
	CSSL-GNI	3.2	26.7	23.6	0.0	75	581	355	0.0
	NIL-APOI	3.0	23.9	20.0	0.1	75	572	389	0.0
Ambient	Koshihikari	3.1	23.2	14.1	0.0	74	554	237	0.0
	CSSL-GNI	3.1	25.3	20.9	0.0	74	559	283	0.0
	NIL-APOI	2.9	22.6	18.0	0.0	69	544	293	0.0
ANOVA									
CO <sub>2</sub> concentration (C)		NS <sup>§</sup>	NS	‡	NS	NS	NS	‡	NS
Genotype (G)		‡	*	**	NS	‡	NS	NS	NS
C × G		NS	NS	NS	NS	NS	NS	NS	NS

**Table 6.** Mean spikelet number and spikelet weight per square meter at each position (primary, secondary, tertiary, and quaternary) in the panicles as affected by different CO<sub>2</sub> concentrations and genotypes averaged for two years (2012 and 2013). \*Significant at P < 0.05. \*\*Significant at P < 0.01. †Means within a column followed by the same lowercase letter do not differ significantly (P < 0.05). ‡Significant at P < 0.10. §Not significant at P < 0.10.

and NIL-APOI, increased atmospheric CO<sub>2</sub> concentrations may increase the tertiary grain weight per spikelet in spite of their large tertiary spikelet number per square meter resulting in an increased percentage of filled spikelets. In a recent study, Koshihikari and Takanari were compared, and Takanari, which has a higher number of spikelets, showed a substantial increase in grain weight of inferior spikelets under FACE conditions, while the weight of superior spikelets was not affected under those conditions<sup>28</sup>. The results of the present study confirm that the increased weight of the inferior spikelets could be enhanced by increased source capacity, and could contribute to a greater yield response to elevated CO<sub>2</sub> conditions. It is worth noting, however, that the source capacity for Takanari was also significantly greater than that for Koshihikari under both current and future CO<sub>2</sub> conditions<sup>29</sup>, suggesting that an increase in source capacity is also needed to meet the increased demand of grain for carbon.

To resolve global food issues, there is a need to develop high-yielding varieties that can adjust to the future environment. Recently developed high-yielding varieties in Japan are divided into two groups, inbred varieties from *indica* and *indica-japonica* varieties (i.e., pureline varieties originated from the cross of *indica* and *japonica* parents). *Indica* high-yielding varieties developed in Japan have higher percentage of filled spikelets than *indica-japonica* high-yielding varieties<sup>30,31</sup>, because they have higher grain weight of tertiary spikelet<sup>30</sup>. This suggests that they are able to produce high levels of carbohydrates and translocate them to their sink. *Indica-japonica* high-yielding varieties have a higher sink capacity than *indica* high-yielding varieties developed in Japan<sup>31</sup>. Thus, such varieties are expected to produce a high grain yield under the increased atmospheric CO<sub>2</sub> concentrations that may occur in the near future.

Lodging reduces grain yield as a result of self-shading and reduced canopy photosynthesis<sup>32</sup>. The results of a previous report indicated that lodging was increased by increasing panicle weight under ambient conditions, but was alleviated under FACE conditions<sup>33</sup>. Consequently, to develop varieties which can contribute a stable and high production of rice under increased atmospheric CO<sub>2</sub> concentrations in the near future, introducing alleles to conventional varieties that enhance sink capacity represents a useful strategy, because the risk of lodging as a results of increasing panicle weight is relatively low.

## Materials and Methods

**Experimental design and crop management.** The study was conducted in 2012 and 2013 on a Fluvisol, which is typical of alluvial areas, at the Tsukubamirai free-air CO<sub>2</sub> enrichment field (35°58'N, 139°60'E, 10 m above sea level), Tsukubamirai, Ibaraki, Japan. Rice was grown previously in the field was rice in both years. Treatments included two atmospheric CO<sub>2</sub> concentrations and three genotypes (one variety and two lines), which were arranged as a split-plot experiment with four replicates in a randomized complete block design. The main plot and subplot were atmospheric CO<sub>2</sub> concentration and genotype, respectively. The mean temperature and

Month	Stage of month	Mean temperature			Solar radiation		
		2012 (°C)	2013 (°C)	Normal <sup>†</sup> (°C)	2012 (MJ m <sup>-2</sup> d <sup>-1</sup> )	2013 (MJ m <sup>-2</sup> d <sup>-1</sup> )	Normal (MJ m <sup>-2</sup> d <sup>-1</sup> )
May	early						
	middle						
	late	19.2	20.1	17.9	21.4	20.1	18.4
June	early	19.9	20.1	19.4	18.5	22.7	17.8
	middle	19.8	22.5	20.2	15.2	10.5	15.5
	late	19.8	21.3	20.9	20.6	17.3	13.4
July	early	22.9	25.6	22.5	17.6	20.0	15.2
	middle	25.8	25.1	23.9	19.6	21.1	15.3
	late	25.8	24.5	25.2	19.4	15.8	17.8
August	early	26.2	26.6	25.8	20.5	19.2	18.1
	middle	26.9	28.4	25.5	17.9	22.6	17.3
	late	27.4	25.9	25.1	21.5	17.1	15.8
September	early	25.4	25.0	24.0	16.1	13.9	14.4
	middle	25.9	23.3	22.0	15.5	16.7	12.3
	late						

**Table 7.** Mean temperature and solar radiation at the Tsukubamirai free-air CO<sub>2</sub> enrichment (FACE) facility, Tsukubamirai, Ibaraki, Japan, during the 2012 and 2013 crop seasons. <sup>†</sup>30-yr average (1981–2010) recorded at the nearest weather station Tateno.

solar radiation recorded during the cropping season in 2012 and 2013 were higher than those in a normal year (Table 7).

The following variety and lines were used: Koshihikari, which is a conventional variety in Japan; a CSSL carrying the *GNI* region from a high-yielding variety, Takanari, in the Koshihikari genetic background (CSSL-*GNI*); and a NIL carrying a favorable allele of *APO1* from Takanari in the Koshihikari background (NIL-*APO1*). CSSL-*GNI* was developed by repeated backcrossing with Koshihikari and marker-assisted selection<sup>16</sup>. NIL-*APO1* was developed in the same way. The selection of the alleles of Takanari were made based on our previous FACE experiment that Takanari showed a greater yield response with greater sink capacity<sup>11</sup>.

Germinated seeds were sown in nursery boxes in late April. Seedlings were transplanted by hand into the paddy field in late May at a density of 22.2 hills m<sup>-2</sup> (three seedlings per hill, 30 cm wide × 15 cm long). About a month before transplanting, plots received 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 100 kg ha<sup>-1</sup> K<sub>2</sub>O in the form of synthetic fertilizer broadcast by hand. One week before transplanting, plots received 120 kg ha<sup>-1</sup> N in the form of synthetic fertilizer (urea/LP100/LP140, 1:2:1) broadcast by hand, with the fertilizer incorporated into the soil by puddling and leveling with a harrow. One month before heading, plants received 40 kg ha<sup>-1</sup> N in the form of synthetic fertilizer (LP40) broadcast by hand. LP40, LP100, and LP140 (JCAM AGRIC. Co., Ltd., Tokyo, Japan) are controlled-release fertilizers, which release 80% of the total N content at a uniform rate up to 40, 100, and 140 days after application, respectively, at 20–30 °C. After trimming, each plot was 1.5-m wide × 2.25-m long. To prevent lodging, a polyethylene netting with a horizontal of 30 cm × 30 cm about 70 cm above the soil surface was installed in each plot. Weeds and pests were controlled with biocides such as those described by Hasegawa *et al.*<sup>11</sup>.

**CO<sub>2</sub> control.** The atmospheric CO<sub>2</sub> concentration was controlled as described by Nakamura *et al.*<sup>34</sup>. The average atmospheric CO<sub>2</sub> concentration ± day-to-day standard deviations during the crop season in FACE plots was 578 ± 15.7 μmol mol<sup>-1</sup> in 2012 and 576 ± 15.5 μmol mol<sup>-1</sup> in 2013, and in ambient plots was 383 ± 11.2 μmol mol<sup>-1</sup> in 2012 and 383 ± 11.4 μmol mol<sup>-1</sup> in 2013.

**Sampling and measurements.** At heading (Zadoks code 59)<sup>35</sup> (early August), plants from 0.405 m<sup>2</sup> (nine hills) were sampled. Two hills with an average panicle number were selected and separated into green leaf blades, dead leaf blades, leaf sheaths plus stems, and panicles. After the area of green leaf blades was measured with a leaf area meter (AAM-9, Hayashi Denko, Tokyo, Japan), each plant part was dried at 80 °C in a ventilated oven for 2 days with the plants of the remaining hills to determine their dry weight. The dried samples were ground to a powder with a vibrating sample mill (TI-1001, CMT, Co., Ltd., Tokyo, Japan) in order to measure nonstructural carbohydrate (NSC) concentrations. Concentrations of NSCs in the leaf sheaths plus stems were determined as described by Ohnishi and Horie<sup>36</sup>.

At maturity (Zadoks code 92)<sup>35</sup> (mid-September), plants from 0.810 and 0.855 m<sup>2</sup> (18 and 19 hills) in 2012 and 2013, respectively, were sampled. Two hills were selected with an average panicle number and were separated into leaf blades, leaf sheaths plus stems, and panicles. Their NSC concentrations were determined as described. The 15 hills were air-dried until they reached a constant weight. The panicles were counted, and the air-dried plants were threshed. Half of the rough rice grains were dehusked to determine the grain weight and the 1000-grain weight. Grain numbers with a thickness of 1.6 mm or more making up 20 g were counted with a multi auto counter (KC-10, Fujiwara Seisakusho, Tokyo, Japan), and the 1000-grain weights were calculated from this value. Grain yield and 1000-grain weight were corrected based on a 150 g kg<sup>-1</sup> moisture concentration. Approximately 100 g of rough rice grains were counted with a multi auto counter (KC-10, Fujiwara Seisakusho, Tokyo, Japan) to determine the spikelet



numbers. The remaining two hills were harvested and air-dried until they reached a constant weight. The spikelet numbers at each position (primary, secondary, tertiary, and quaternary) in the three panicles with greater weights were recorded as described in Fig. 1, and then the weights of the spikelets were determined.

**Statistical analysis.** Statistical analyses were performed using a general linear model in SPSS (SPSS 17.0, SPSS Inc., Chicago, IL, USA). CO<sub>2</sub> concentration and genotype were considered as fixed effects. Year and replication were considered as random effects. Analysis of variance (ANOVA) was conducted to test the effects of CO<sub>2</sub> concentration and genotype on yield, its components, and panicle structure. Significant treatment effects ( $P < 0.05$ ) were explored using Fisher's protected least significant difference (LSD).

## References

- Porter, J. R. *et al.* Food security and food production systems. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. Field, C. B. *et al.*) 485–533 (Cambridge University Press, 2014).
- Earth Systems Research Laboratory. Global Monitoring Division, National Oceanographic and Atmospheric Administration, US Department of Commerce. *Trends in atmospheric carbon dioxide*. <http://www.esrl.noaa.gov/gmd/ccgg/trends/> (Date of access: 15/03/2017) (2016).
- FAO, *Global agriculture towards 2050*. [http://www.fao.org/fileadmin/templates/wfs/docs/Issues\\_papers/HLEF2050\\_Global\\_Agriculture.pdf](http://www.fao.org/fileadmin/templates/wfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf) (Date of access: 15/03/2017) (2009).
- GriSP. *Rice almanac*. 4th edn (IRRI, 2013).
- Horie, T. *et al.* Can yields of lowland rice resume the increases that they showed in the 1980s? *Plant Prod. Sci.* **8**, 259–274 (2005).
- Kimball, B. A., Kobayashi, K. & Bindi, M. Responses of agricultural crops to free-air CO<sub>2</sub> enrichment. *Adv. Agron.* **77**, 293–368 (2002).
- Long, S. P., Ainsworth, E. A., Rogers, A. & Ort, D. R. Rising atmospheric carbon dioxide: plants FACE the future. *Annu. Rev. Plant Biol.* **55**, 591–628 (2004).
- Kim, H. Y. *et al.* Effects of free-air CO<sub>2</sub> enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crop. Res.* **83**, 261–270 (2003).
- Yang, L. *et al.* The impact of free-air CO<sub>2</sub> enrichment (FACE) and N supply on yield formation of rice crops with large panicle. *Field Crop. Res.* **98**, 141–150 (2006).
- Shimono, H. *et al.* Genotypic variation in rice yield enhancement by elevated CO<sub>2</sub> relates to growth before heading, and not to maturity group. *J. Exp. Bot.* **60**, 523–532 (2009).
- Hasegawa, T. *et al.* Rice cultivar responses to elevated CO<sub>2</sub> at two free-air CO<sub>2</sub> enrichment (FACE) sites in Japan. *Funct. Plant Biol.* **40**, 148–159 (2013).
- Zhang, G. *et al.* The effects of free-air CO<sub>2</sub> enrichment (FACE) on carbon and nitrogen accumulation in grains of rice (*Oryza sativa* L.). *J. Exp. Bot.* **64**, 3179–3188 (2013).
- Ashikari, M. *et al.* Cytokinin oxidase regulates rice grain production. *Science* **309**, 741–745 (2005).
- Terao, T., Nagata, K., Morino, K. & Hirose, T. A gene controlling the number of primary rachis branches also controls the vascular bundle formation and hence is responsible to increase the harvest index and grain yield in rice. *Theor. Appl. Genet.* **120**, 875–893 (2010).
- Ohsumi, A. *et al.* Evaluation of yield performance in rice near-isogenic lines with increased spikelet number. *Field Crop. Res.* **120**, 68–75 (2011).
- Takai, T. *et al.* Genetic mechanisms underlying yield potential in the rice high-yielding cultivar Takanari, based on reciprocal chromosome segment substitution lines. *BMC Plant Biol.* **14**, 295 (2014).
- Leakey, A. D. B. *et al.* Elevated CO<sub>2</sub> effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* **60**, 2859–2876 (2009).
- Usui, Y. *et al.* Rice grain yield and quality responses to free-air CO<sub>2</sub> enrichment combined with soil and water warming. *Glob. Change Biol.* **22**, 1256–1270 (2016).
- Yoshida, S. *Fundamentals of rice crop science* (IRRI, 1981).
- Sumi, A., Hakoyama, S., Weng, J. H., Agata, W. & Takeda, T. Analysis of plant characteristics determining ear weight increase during the ripening period in rice (*Oryza sativa* L.). II. The role of the reserved carbohydrate at heading stage upon the receptive efficiency of assimilation products in spikelets. *Jpn. J. Crop Sci.* **65**, 214–221 (1996).
- Laza, R. C., Peng, S. B., Akita, S. & Saka, H. Contribution of biomass partitioning and translocation to grain yield under sub-optimum growing conditions in irrigated rice. *Plant Prod. Sci.* **6**, 28–35 (2003).
- Gendua, P. A., Yamamoto, Y., Miyazaki, A., Yoshida, T. & Wang, Y. L. Responses of yielding ability, sink size and percentage of filled grains to the cultivation practices in a Chinese large-panicle-type rice cultivar, Yangdao 4. *Plant Prod. Sci.* **12**, 243–256 (2009).
- Takai, T. Rice yield potential is closely related to crop growth during late reproductive period. *Field Crop. Res.* **96**, 328–335 (2006).
- Kobata, T., Yoshida, H., Masiko, U. & Honda, T. Spikelet sterility is associated with a lack of assimilate in high-spikelet-number rice. *Agron. J.* **105**, 1821–1831 (2013).
- Kato, H. *et al.* Breeding of the rice cultivar “Leaf Star” with high stem and leaf yield for whole crop silage. *Bull. Natl. Inst. Crop Sci.* **11**, 1–15 (2010).
- Matsushita, K. *et al.* ‘Tachisuzuka’, a new rice cultivar with high straw yield and high sugar content for whole-crop silage use. *Breed. Sci.* **61**, 86–92 (2011).
- Terai, K. Correlation of nodal position of spikelet in panicle and date of anthesis with the grain ripening of rice. *Jpn. J. Crop Sci.* **63**, 34–41 (2008).
- Zhang, G. *et al.* Grain growth of different rice cultivars under elevated CO<sub>2</sub> concentrations affects yield and quality. *Field Crop. Res.* **179**, 72–80 (2015).
- Chen, C. P. *et al.* Do the rich always become richer? Characterizing the leaf physiological response of the high-yielding rice cultivar Takanari to free-air CO<sub>2</sub> enrichment. *Plant Cell Physiol.* **55**, 381–391 (2014).
- Nakano, H. & Tsuchiya, S. Yield response to cultivar and sowing pattern in high-yielding rice. *Crop Sci.* **52**, 2800–2806 (2012).
- Yoshinaga, S., Takai, T., Arai-Sanoh, Y., Ishimaru, T. & Kondo, M. Varietal differences in sink production and grain-filling ability in recently developed high-yielding rice (*Oryza sativa* L.) varieties in Japan. *Field Crop. Res.* **150**, 74–82 (2013).
- Setter, T. L., Laureles, E. V. & Mazaredo, A. M. Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. *Field Crop. Res.* **49**, 95–106 (1997).
- Shimono, H. *et al.* Lodging in rice can be alleviated by atmospheric CO<sub>2</sub> enrichment. *Agric. Ecosyst. Environ.* **118**, 223–230 (2007).
- Nakamura, H. Performance of the enlarged Rice-FACE system using pure CO<sub>2</sub> installed in Tsukuba, Japan. *J. Agric. Meteorol.* **68**, 15–23 (2012).
- Zadoks, J. C., Chang, T. T. & Konzak, C. F. A decimal code for the growth stages of cereals. *Weed Res.* **14**, 415–421 (1974).
- Ohnishi, M. & Horie, T. A proxy analysis of nonstructural carbohydrate in rice plant by using the gravimetric method. *Jpn. J. Crop Sci.* **68**, 126–136 (1999).

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## Author Contributions

H.N., S.Y., T.H. and M.K. designed the experiment. H.N., S.Y., T.T., Y.A.-S., H.S., T.T., Y.U., H.N., T.H. and M.K. performed the experiment. K.K. and T.Y. developed NIL-*APO1*. H.N. analyzed the results. H.N., T.H. and M.K. wrote the manuscript. S.Y., T.T., Y.A.-S., K.K., T.Y., H.S., T.T., Y.U. and H.N. provided advise on the manuscript.

## Additional Information

**Competing Interests:** The authors declare that they have no competing interests.

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