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Rapid Report

The effects of *PIK3CA* and *TP53* on prognosis in patients with Triple-negative breast cancer (TNBC): a single institution's long-term follow-up (6 years)

Yuan Zhao[1,2], Wenru Tang[3], Limei Sun[2], Dun Xiong[2], Miaomiao Sheng[3,*], Ying Luo[3,*]

[1] *Faculty of Life Science and Technology, Kunming University of Science and Technoloy, 650500 Kunming, Yunnan, China*

[2] *Department of Oncology, The Affiliated Hospital of Kunming University of Science and Technology, 650500 Kunming, Yunnan, China*

[3] *Laboratory of Molecular Genetics of Aging & Tumor, Medical School, Kunming University of Science and Technology, 650500 Kunming, Yunnan, China*

*Correspondence: luoying@gmc.edu.cn (Ying Luo); shengmm@kust.edu.cn (Miaomiao Sheng)

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Objectives: This study investigated the prognostic value of *PIK3CA* and *TP53* during a 6-year follow-up of breast cancer patients. We also analyzed the impact of other factors on progression-free survival (PFS) and patients' overall survival (OS). *Methods:* The expression of estrogen receptor (ER), progesterone receptor (PR), and Ki67 in cancer tissues was detected by immunohistochemistry. For each patient, Epidermal Growth Factor Receptor 2 (HER2 was evaluated using fluorescence *in situ* hybridization (FISH). Sanger sequencing was used to detect hotspot mutations in *PIK3CA* and *TP53* genes. *Results*: Lymph node metastasis is the most important factor affecting the patient's prognosis (74.3% vs. 81.6%, $p = 0.04$). The mutation rates of *PIK3CA* and *TP53* in Triple-negative breast cancer (TNBC) patients were significantly higher than those of other breast cancer types (29.4% vs. 3.6%, $p = 0.002$). In TNBC, the PFS of patients with *PIK3CA* and *TP53* mutations is poorer than non-carriers (0% vs. 75%, $p = 0.003$). The OS of breast cancer patients in Yunnan Province is lower than that of regions with rapid economic development in China (78.08% vs. 90.5%). *Conclusions*: Our results indicate that *PIK3CA* and *TP53* mutations are poor prognostic factors for patients with TNBC. *PIK3CA* and *TP53* mutations could be used as a predictor for prognosis in TNBC patients.

Keywords

PIK3CA; *TP53*; Breast cancer; Survival

1. Introduction

In recent years, breast cancer (BC) incidence has rapidly increased. According to the latest global cancer report, BC has become one of the leading lethal factors among females [1]. *PIK3CA* and *TP53* are the genes highly related to BC [2–4]. Phosphatidylinositol 3-kinase catalytic subunit α gene was first used by Volinia to locate the *3q26.3* gene by *in situ* hybridization technology [5]. It contains 20 exons and encodes the p110a catalytic subunit of class I P13K. About 80%–90% of *PIK3CA* gene mutations are concentrated in the 9th and 20th exon of the gene, corresponding to the helical region and the kinase region of the enzyme. It is a non-random high-frequency mutation that shows its importance in tumorigenesis.

The wild-type *TP53* gene is a tumor suppressor gene with extensive tumor suppressor effect. Its main functions include: inducing cell cycle arrest; promoting DNA repair and cell apoptosis, thereby avoiding the accumulation of damaged DNA; maintaining the genome's stability; regulating cell differentiation and senescence; and inhibiting tumor vascular proliferation. The protein expressed by wild-type *TP53* is a very important apoptosis-inducing protein, but the p53 protein encoded by the *TP53* in normal cells has a short half-life of about 20 minutes. It has a low content so it is difficult to detect by immunohistochemical methods. The mutant *TP53* is a cancer-promoting gene [6]. The frequency of mutations varies with the histologic and biochemical characteristics of the type of breast cancer, being more common in ductal vs. lobular, lymph-node positive vs. lymph-node negative, ER-negative vs ER-positive, and HER2-positive vs HER2-negative cases [7]. Evidence suggests that 95.1% of the *TP53* point mutations in tumors mainly occur at the highly conserved positions 175, 245, 248, 249, 273, and 282 [8]. The occurrence of this mutation causes p53 to suppress cancer with the loss of this activity promoting the malignant transformation of cells, thereby transforming tumor suppressor genes into oncogenes [9]. Due to changes in the protein's primary structure, the transformation and metabolism of the p53 protein in the cell slows down and its half-life is significantly prolonged. It can be detected by immunohistochemistry for a range of 2 to 12 hours. Therefore, the p53 protein detected by the immunohistochemical method is the protein expressed by the mutant *TP53*, which is a cancer-promoting factor. The *TP53* mutation occurs in 30–35% of invasive primary BC.

BC's incidence is positively correlated with the national economic level; it is much higher in developed countries than in moderately developed countries. However, most BC deaths occur in middle-income countries because most women in those countries cannot get regular health checks or afford expensive targeted drugs [10]. In China, BC sur-

vival rates vary widely from region to region. In this study, we collected 73 cases of breast cancer in Yunnan province, an underdeveloped area in southwest China, and conducted long-term 6+ year follow-up to study the related factors of PFS and OS.

2. Methods

2.1 Patients and tissue samples

Seventy-three BC patients were selected from 2013 to 2014 in Yunnan province in southwest China. All patients were treated by the Breast Cancer Diagnosis and Treatment Guidelines formulated by the Chinese Society of Clinical Oncology, 2014 edition, China. The follow-up time was 84 months to 96 months, with an average of 6.5 years. The clinical data of all patients collected from the hospital records include age, tumor size, and lymph node metastasis (**Supplementary Table 1**). The Affiliated Hospital approved this study of Kunming University of Science and Technology (Approval number: 2013PYICE006). All enrolled patients signed the informed consent form for this study.

2.2 Immunohistochemistry

KUST Aging and Tumor Molecular Laboratory tested the expression levels of ER, PR, Ki67, and TP53, mutations of *PIK3CA*, and *TP53*. Positive localization of ER, PR, and *TP53* is in the nucleus. The positivity of ER, PR and *TP53* with more than 1% of tumor nuclei are brown or tan. Patients with Ki67 $\leq$20% are considered low expression and Ki67 higher than 20% is considered high expression.

2.3 Fluorescence in situ hybridization

The patient's Human Epidermal Growth Factor Receptor 2 (HER2) status was based on the Fluorescence *In Situ* Hybridization (FISH) results. The FISH experiment was carried out with Hercep Test For the Automated Link Platform Kit in FFPE samples, and 20 cells from each region were selected for analysis. In the count 20 tumor cells, orange represents HER2 signal, and the green represents CEP17 signal. HER2/CEP17 $\geq$2.0 suggests that HER2 amplification is positive. HER2/CEP17 $<$2.0 with the average HER2 gene copy number $\geq$6.0 indicates that HER2 amplification is positive. Meanwhile, HER2/CEP17 $<$2.0 with the average copy number of HER2 gene $<$4.0, suggests negative HER2 gene amplification. When the HER2/CEP17 ratio is less than 2.0 and the average HER2 gene copy number is greater than or equal to 4.0 and less than 6.0, the HER2 amplification is uncertain.

2.4 Sanger sequencing

According to the manufacturer's instructions, DNA was extracted from FFPE breast tumor specimens using the QIAamp DNA FFEP Tissue Kit with design primers for exons 5–8 of *TP53* and exons 9 and 20 of *PIK3CA* (**Supplementary Table 2**). All fragments were sequenced using the BigDye Terminator Cycle Sequencing Kit and ABI7500 automated sequencer (Applied Bio-systems). Independent PCRs and Sanger sequencing confirmed that each mutation is duplicate.

Table 1. Basic clinical information of the 73 patients with breast cancer.

Characteristic	Overall cohort (n = 73)
	n (%)
Age (y) Median (range)	49 (24–74)
Tumor size (cm)	
$\leq$2	45 (62%)
$>$2	28 (38%)
Molecular classification	
luminal A	13 (18%)
luminal B	35 (48%)
HER2 overexpression	8 (11%)
Basal-like	17 (23%)
Lymph node	
Negative	35 (48%)
Positive	38 (52%)
ER	
Negative	47 (64%)
Positive	26 (36%)
PR	
Negative	41 (56%)
Positive	32 (44%)
HER2	
Negative	32 (44%)
Positive	41 (56%)
Ki67 high expression	
Negative	36 (49%)
Positive	37 (47%)
TP53 expression	
Negative	56 (77%)
Positive	17 (23%)
TP53 mutation	
Negative	63 (86%)
Positive	10 (14%)
PIK3CA mutation	
Negative	37 (51%)
Positive	36 (49%)

2.5 Statistics

Statistical analysis was performed using the SPSS 20.0 software (IBM Corp., Chicago, IL, USA). OS and PFS curves were calculated by the Graphpad Prism software version 6.0 (GraphPad Software Inc., San Diego, CA, USA) and compared by long-rank tests. A p-value $<$ 0.05 was considered significant.

3. Results

The basic clinical information for the patients included in this study is shown in Table 1. As shown in Fig. 1, *PIK3CA* mutation was significant and positively correlated with tumor size ($p < 0.05$). Lymph node metastasis was highly correlated with PR expression ($p < 0.05$) while the expression of ER is highly correlated with PR and HER2 ($p < 0.05$).

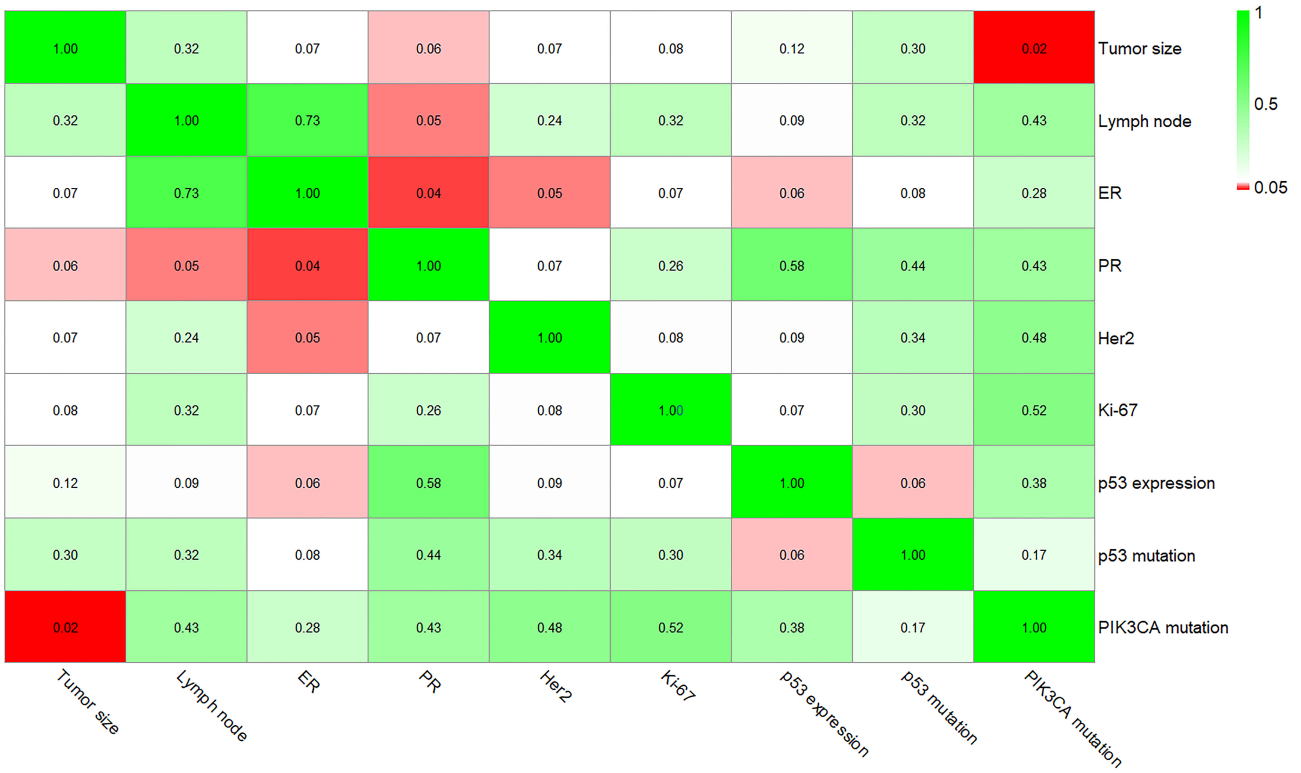

Fig. 1. Correlation between clinical data. *PIK3CA* and tumor size, lymph node metastasis and PR expression, ER expression and PR expression, ER expression and HER2 are highly correlated ($p < 0.05$).

The *PIK3CA* positive rates of different types of BC are Luminal A (31%), Luminal B (43%), HER2+ (50%), and TNBC (53%). The *TP53* positive rates of different types of BC are Luminal A (15%), Luminal B (7%), HER2+ (38%), and TNBC (30%). The patients' co-mutation of *TP53* and *PIK3CA* in TNBC is higher than that of other types (29.4% vs. 3.6%, $p = 0.002$) (**Supplementary Table 3**).

Lymph node metastasis has a greater correlation with the OS of BC patients. The OS of patients with lymph node metastasis is lower than that of patients without lymph node metastasis (74.3% vs. 81.6%, $p = 0.04$) (Fig. 2).

By the last telephone follow-up, five patients with *PIK3CA* and *TP53* mutations in TNBC had recurrence or metastases. We found that *TNBC* patients with *PIK3CA* and p53 mutations have shorter PFS than patients without these two mutations (Fig. 3).

4. Discussion

Data on BC rates in economically developed areas are easier to obtain [11], but these data are relatively scarce in economically underdeveloped areas.

In the United States, BC survival rate continues to rise, and the 5-year relative survival rate of early BC has reached 90%–99% [12]. The 5-year survival rate of BC patients in this study was 78.08%, similar to survival observed 30 years ago in developed countries [13], which is lower than that of developed countries [14, 15] and other developed regions in China (78.08% vs. 90.5%) [16]. However, it is higher than in most countries in sub-Saharan Africa [17].

Lymph nodes were an important predictor of survival. Leonel *et al.* [18] retrospectively analyzed 1711 breast cancer patients, and the results showed that the prognosis of BC patients is closely related to lymph node metastasis but has nothing to do with the number of lymph nodes involved. This is similar to our research findings.

There was no significant difference in PFS and OS of patients with different histologic types of breast cancer. This may be due to the small number of patients studied and insufficient follow-up time. To this end, we are continuing to enroll patients and continue telephone follow up with patients.

In this study, we found that the mutation rate of *PIK3CA* and *TP53* in TNBC patients is higher than that of HR+ breast cancer patients and is significantly related to the shortening of PFS. However, according to the results of other studies, *PIK3CA* mutations occur less frequently in TNBC compared with HR+ and HER2+ type [19]. Saal *et al.* [20] reported a significant positive association between *PIK3CA* mutations and HER2 overexpression, suggesting that more than one mechanism is involved in activating the negative effect of PI3K/AKT/mTOR signaling and is associated with poor prognosis in metastatic breast cancer. In the present study, we failed to show a significant association between HER2 amplification and *PIK3CA* mutations. TNBC is a type of BC with a high recurrence rate and a poor prognosis, accounting for

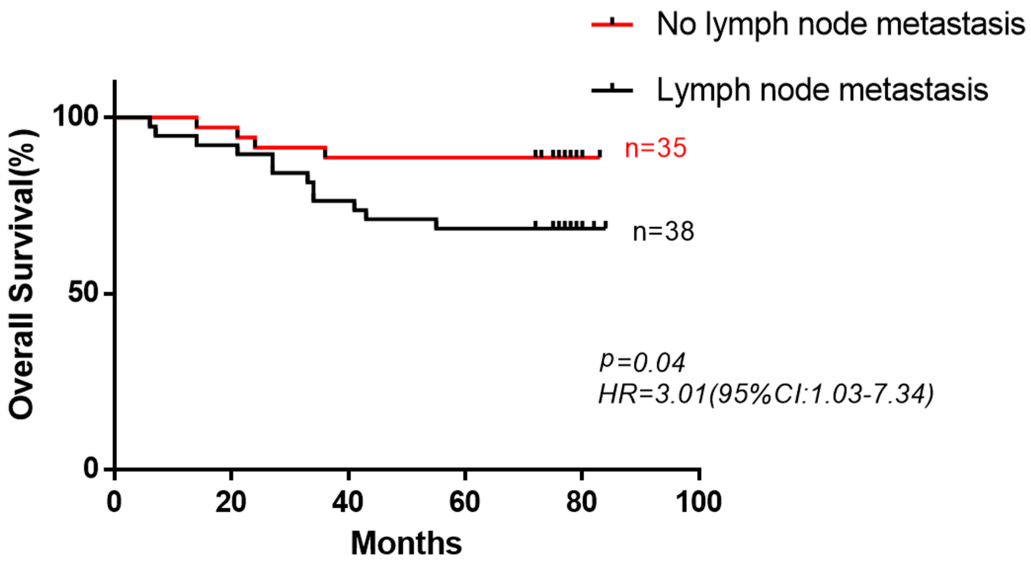

Fig. 2. Lymph node metastasis is a predictor of poor prognosis. Whether lymph node metastasis has a greater impact on the BC patient's OS ($p = 0.04$).

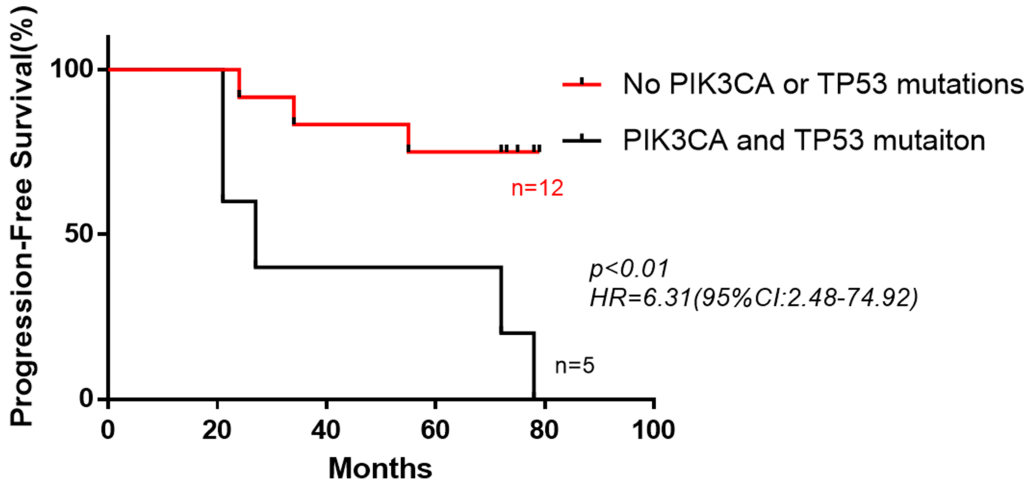

Fig. 3. PFS of TNBC with or without *PIK3CA* and *TP53* mutations. The appearance of *PIK3CA* and *TP53* mutations heralds the poor prognosis of TNBC.

15%–20% of breast cancers. With the absence of the expression of ER, PR, and HER2 in TNBC, endocrine therapy and HER2 targeted therapy cannot be used to treat TNBC. Compared with other types of BC, TNBC has a higher sensitivity to chemotherapy drugs.

The mutation of *PIK3CA* gene is most common in breast cancer along with *TP53* tumor suppressor gene. One of the genetic mutations can occur in more than 40% of breast cancer patients [21]. Although the mutations of *PIK3CA* gene were scattered in most exons, there were three hot spots, namely e542k, e545k and h1047r. E542k and e545k were located in the helix region of *PIK3CA* gene exon 9, while h1047r was located in the kinase region of exon 20. This study found that the mutation of *PIK3CA* gene is related to the resistance of breast cancer patients to anticancer drugs trastuzumab and rapatinib [22]. The mutation of *PIK3CA* gene is correlated with the prognosis of breast cancer [23]. All these indicate the important role of *PIK3CA* gene mutation in breast cancer. The mutation rates of *PIK3CA* and *TP53* in Triple-negative breast cancer (TNBC) patients were significantly higher than in those of other types. In TNBC, the PFS of patients with *PIK3CA* and *TP53* mutations is lower than in those with non-mutations. Pathogenic mutations in *PIK3CA* will cause it to encode abnormal p110a subunits, keep the P13K enzyme in a continuous activation state, enhance intracellular signal transduction, and cause cells to proliferate uncontrollably and form tumors [24]. PI3K/AKT/mTOR signaling pathway is related to the pathogenesis of TNBC [25]. Mutations in these two genes may lead to a poorer prognosis for breast cancer. Besides, *PTEN* and *INPP4B* phosphatase are frequently lost in TNBC. The inactivation of *PTEN* in animal models can lead to "basic-like" BC. Therefore, researchers have been working to develop drugs that target PI3K more precisely to improve BC treatment. Alpelisib, combined with fulvestrant, is

now available for postmenopausal women with advanced or metastatic hormone receptor(HR)-positive, HER2-negative, PIK3CA-mutated BC [26]. Besides, promising data have been observed in randomized phase II trials of AKT inhibitors combined with fulvestrant or paclitaxel in metastatic HR-positive, HER2-negative disease, and Triple-negative breast cancer (TNBC) [27]. A further study has suggested that aspirin can prolong the metastasis time of breast cancer patients with *PIK3CA* mutation [28].

PTEN gene is composed of 9 exons and encodes a protein of 403 amino acids. It has dual specificity phosphatase activity. The decrease of PTEN expression level is related to the occurrence of malignant tumors. *PTEN*, as phosphatase, can inhibit the PI3K/Akt pathway. It can activate capase-3 and capase-9 to promote apoptosis while also it can activate bad and Bcl-2 family to resist apoptosis; It also prevents the release of cytochrome C from mitochondria and promotes the inactivation of transcription factors. *PTEN/PI3K* can regulate the concentration of PIP3 and maintain the balance of Akt activity. Inactivation of *PTEN* will lead to the loss of function of PTEN encoded protein and inhibition of *PI3K/Akt*.

In BC, the prevalence of *TP53* mutations depends on the molecular subtype of the disease, being present in approximately 80% of patients with the TNBC, 10% of samples from patients with luminal A disease, 30% of those with luminal B type, and 70% of those with HER2+ BC. Because of this high prevalence, mutated p53 is a potential biomarker and therapeutic target for BC patients, especially those with the TN subtype [29]. Many studies have shown that the mutation of *TP53* predicts the poor prognosis for breast cancer [30, 31]. The mutation of *TP53* leads to the loss of the function of its tumor suppressor gene and leads to malignant expression [28]. Nevertheless, *TP53* has not been considered a target for drug therapy. This view is beginning to change as some compounds have recently been shown to reactivate mutant *TP53*, restore its wild-type properties, and mediate the anti-cancer activity of mutant p53 expressed in preclinical tumor models. Some of these mutant compounds, such as 3-quinuclidine, PRIMA-1, APR-246, PK11007, and COTI-2 have been studied for their potential anti-cancer activity BC models [32].

Singh *et al.* [33] and Astanehe *et al.* [34] reported that *PIK3CA* mutations and *TP53* mutations were mutually exclusive. Differing from their result, we have not found a significant association between *PIK3CA* mutations and P53 mutation. In this study, the mutation rate of *PI3K* gene (29.4%) was higher than that of *TP53* gene (3.6%). These results indicate that *PI3K/Akt* pathway plays an important role in the development of breast cancer. In addition, the PFS and OS of breast cancer patients with *PIK3CA* and *TP53* gene mutations were significantly lower than those without mutations, suggesting that gene mutation is one of the predictors of poor prognosis in breast cancer patients. In conclusion, our results suggested that patients with *PIK3CA* and *P53* mutation present were a disadvantaged and unfavorable group of patients during a long follow-up. Further research is needed to study how to use these molecular biomarkers to identify patients who do not require chemotherapy but may be effective for targeted therapy. This may stimulate physicians to advise *PIK3CA* and *TP53* mutation screening in clinical practice to avoid unnecessary treatment. Besides, in economically underdeveloped areas, regular physical examinations and standardized treatment are the most important means to improve the OS of BC patients.

Author contributions

YZ and WT collected the samples and performed the research. LS and DX provided help on the experiments. MS and YL analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Medical College of Kunming University of science and technology.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://ejgo.imrpress.com/EN/10.31083/j.ejgo4206191.

Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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