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# Expression pattern of *CRYAB* and *CTGF* genes in two pig breeds at different altitudes

[*Padrão de expressão dos genes CRYAB e CTGF em duas raças de suínos em altitudes diferentes*]

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## ABSTRACT

Tibetan pigs are characterized by significant phenotypic differences relative to lowland pigs. Our previous study demonstrated that the genes *CRYAB* and *CTGF* were differentially expressed in heart tissues between Tibetan (highland breed) and Yorkshire (lowland breed) pigs, indicating that they might participate in hypoxia adaptation. *CRYAB* (αB-crystallin) and *CTGF* (connective tissue growth factor) have also been reported to be associated with lung development. However, the expression patterns of *CRYAB* and *CTGF* in lung tissues at different altitudes and their genetic characterization are not well understood. In this study, qRT-PCR and western blot of lung tissue revealed higher *CRYAB* expression levels in highland and middle-highland Tibetan and Yorkshire pigs than in their lowland counterparts. With an increase in altitude, the expression level of *CTGF* increased in Tibetan pigs, whereas it decreased in Yorkshire pigs. Furthermore, two novel single-nucleotide polymorphism were identified in the 5′ flanking region of *CRYAB* (g.39644482C>T and g.39644132T>C) and *CTGF* (g.31671748A>G and g.31671773T>G). The polymorphism may partially contribute to the differences in expression levels between groups at the same altitude. These findings provide novel insights into the high-altitude hypoxia adaptations of Tibetan pigs.

Keywords: *CRYAB* gene, *CTGF* gene, Gene expression, Polymorphism, Pig, Hypoxia adaptation

## RESUMO

*Porcos tibetanos são caracterizados por diferenças fenotípicas significativas em relação aos porcos de planície. Nosso estudo anterior demonstrou que os genes CRYAB e CTGF eram expressos diferentemente nos tecidos do coração entre os porcos tibetanos (raça das terras altas) e Yorkshire (raça das terras baixas), indicando que eles poderiam participar da adaptação à hipoxia. CRYAB (aB-crystallin) e CTGF (fator de crescimento do tecido conjuntivo) também foram relatados como estando associados ao desenvolvimento pulmonar. Entretanto, os padrões de expressão do CRYAB e CTGF nos tecidos pulmonares em diferentes altitudes e sua caracterização genética não são bem compreendidos. Neste estudo, o qRT-PCR e a mancha ocidental de tecido pulmonar revelou níveis de expressão de CRYAB mais elevados em porcos tibetanos e Yorkshire de altitude e média altitude do que em seus pares de planície. Com um aumento na altitude, o nível de expressão do CTGF aumentou nos porcos tibetanos, enquanto diminuiu nos porcos Yorkshire. Além disso, foram identificados dois novos polimorfismos de um único nucleotídeo na região flanqueadora de CRYAB (g.39644482C>T e g.39644132T>C) e CTGF (g.31671748A>G e g.31671773T>G). O polimorfismo pode contribuir parcialmente com as diferenças nos níveis de expressão entre grupos a uma mesma altitude. Estas descobertas proporcionam novos conhecimentos sobre as adaptações de hipoxia a alta altitude dos porcos tibetanos.*

*Palavras-chave: gene CRYAB, gene CTGF, Expressão gênica, Polimorfismo, Porco, Adaptação da hipoxia*

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## INTRODUCTION

The Tibetan pig (TP) mainly lives on the Qinghai-Tibet Plateau, which has an average elevation of 2500–4300-m above sea level. The TP has adapted to harsh conditions such as hypoxia, which makes it a good model for investigating molecular mechanisms of hypoxia adaptation (Liu *et al*., 2018). Our previous study showed that the genes *CRYAB* and *CTGF* were differentially expressed in the heart tissue of Tibetan pigs (TPs; highland breed) and Yorkshire pigs (YYs; lowland breed). In highland, both *CRYAB* and *CTGF* expression were more significant in TP than that in YY, while in lowland *CRYAB* mRNA level is significantly higher in TP than that in YY (Zhang *et al*., 2017). Besides the altitude increasing, both CRYAB and CTGF is significantly increased in TP, while only CRYAB is significantly increased in YY based on RNA-seq evidence (Zhang *et al*., 2017). *CRYAB* (αB-crystallin, also known as *HspB*) is a small heat shock protein that acts primarily as a chaperone to block the aggregation of denatured proteins and prevent cells from stress injury (Bellaye *et al*., 2014; Christopher *et al*., 2014; Reddy and Reddy, 2016). *CRYAB* was also reported to function in mitochondrial pathway of apoptosis during myocardial infarction. (Mitra *et al*., 2013). Connective tissue growth factor (*CTGF*), also known as *CCN2*, is a connective tissue growth factor that is expressed downstream of *TGF-β1*; it leads to the differentiation of mesenchymal cells, formation of collagen, repair of epithelial damage caused by environmental stress, and promotion of angiogenesis and cell proliferation (Allen and Spiteri, 2002; Kosmider *et al*., 2011). *CTGF* was also found to be associated with hypoxia adaptation possibly limiting *VEGF* activation and inhibiting tissue angiogenesis in TPs hearts (Tang *et al*., 2011). Additionally, *CRYAB* and *CTGF* are strongly expressed in patients with idiopathic pulmonary fibrosis (Bellaye *et al*., 2014; Chen *et al*., 2018). The lung is the main organ to resist environmental stress, and hypoxia inducible factor (*HIF-1*) is involved in the pathogenesis of pulmonary fibrosis (Ueno *et al*., 2011). Combining the functional evidence of CRYAB and CTGF in heart under hypoxia and their role in lung function, we consider they are likely to participate in lung physiological process under hypoxia. However, the expression patterns of *CRYAB* and *CTGF* in the lung tissue at different altitudes and their genetic characterizations are not well understood. In this study, the mRNA and protein expression levels of *CRYAB* and *CTGF* in the lung tissue of TPs and YYs in lowland, middle-highland, and highland regions were investigated. Further, genetic variations in the 5′ flanking region of these two genes were identified and their potential binding transcription factors were predicted. The results of this study will provide novel insights into the high-altitude hypoxia adaptations of TPs.

## MATERIALS AND METHODS

The present study was performed in 2020, in the Provincial and Ministerial co-founded collaborative innovation center for R & D in Tibet characteristic Agricultural and Animal Husbandry resources, Tibet, China.

Genomic DNA was extracted from ear tissues of TPs (n = 55) and YYs (n = 45) (Tibet Linzhi, China). Eighteen pigs—TP (n = 9) and YY (n = 9)—from the lowland, Beijing (100-m above mean sea level), eighteen pigs—TP (n = 9) and YY (n = 9)—from the middle-highland, Linzhi of Tibet (2900-m above mean sea level), and eighteen pigs—TP (n = 9) and YY (n = 9)—from the highland, Gongbujiangda of Tibet (3700-m above mean sea level), were used. The pigs used in this experiment were all 7-month-old healthy boars, and TPs and YYs were randomly selected for slaughter and sampling. The pigs in the group were unrelated and in the same feeding environment (except for altitude, the temperature and humidity were kept the same), and they were guaranteed to use the same feed and immunization steps. Lung tissue was collected from each individual, immediately frozen in liquid nitrogen upon collection, and stored at −80 °C until use. All procedures were performed in strict accordance with the protocol approved by the Animal Welfare Committee of Tibet Agriculture and Animal Husbandry University (Permit Number: TPLAB-2016-08-10).

Genomic DNA was extracted from the ear tissue of pigs using the phenol-chloroform extraction method (Sambrook and Fussell, 2001). DNA was dissolved in an appropriate amount of Tris-EDTA buffer solution (pH 8.0) and stored at -20 °C. Total RNA was extracted using the Trizol method (Takara Bio Lnc, China). The concentration and integrity of the RNA samples were determined using agarose gel electrophoresis and measured

using the Nanodrop 2000 Biophotometer (Thermo Fisher Scientific Inc., West Palm Beach, FL, USA). Total protein was isolated in RIPA lysis buffer (Beyotime Ltd., Shanghai, China), stored at -80 °C, and quantified using a BCA protein quantity kit (Beyotime Ltd., Shanghai, China).

Primers were designed for amplification of the 5′ flanking region of the pig *CRYAB* (NC_010451.4) and *CTGF* (NC_010443.5) genes (Table 1) using Primer Premier 5.0 software (Premier Biosoft International, CA, USA). The PCR protocol included an initial denaturation step (95 °C for 3 min) followed by 36 cycles of denaturation at 95 °C for 10 s, annealing at a specific temperature for 30 s (56 °C–60 °C for the different primers listed in Tab. 1), and extension at 72 °C for 1 min. The PCR products from 10 random samples of both TP and YY were pooled and sequenced to identify SNPs between the two breeds using Chromas Pro software 2.1.3 (Technelysium Pty Ltd., Brisbane, Australia). The SNP genotypes were identified via PCR sequencing of each individual. Transcription factor binding sites were predicted using the webserver http://jaspar.binf.ku.dk/.

Table 1. Primers for screening the 5′ flanking region of *CRYAB* and *CTGF* genes

| Primer name | Target region | Primer sequence (5′–3′) | Product size (bp) | Annealing temperature (℃) |
|---|---|---|---|---|
| *CRYAB-1* | -3479/-2698 | F: AAGGTTACTGGGCGGATTCA<br>R: TCCACTGAAAATGGTCTCCG | 781 | 57 |
| *CRYAB-2* | -2893/-1794 | F: GCATTTAGTCTTGGATTGGC<br>R: CAGATGAGGTGACCGTGAG | 1099 | 57 |
| *CRYAB-3* | -2011/-1463 | F: ACCTCTGTGTCCAAATGTCG<br>R: ATTTCCCTTCTCAGTCCCCT | 548 | 57 |
| *CTGF-1* | -2936/-2202 | F: TCATCATAAAGCCCCAACCT<br>R: GGGAAGCCACAGTGAAATCT | 733 | 57 |
| *CTGF-2* | -2484/-1742 | F: GCGTCACTCAACTGTAACCCG<br>R: GGCACCACAATCGGAAAAAA | 850 | 57 |
| *CTGF-3* | -1921/-1181 | F: TGATGTGGTCTGTGCTGAAG<br>R: GGTTTCTTTGGGAGAGGTG | 740 | 57 |
| *CTGF-4* | -1307/-290 | F: GGGGTTCAGTGTTCTTGTTA<br>R: CCTACACAAACAGGGACATT | 1017 | 57 |
| *CTGF-5* | -506/49 | F: AATCAGGAGTGGTGCGAAGA<br>R: CGAGCAGGAGCACGAAG | 558 | 57 |

cDNA was synthesized using a cDNA Synthesis SuperMix Kit (Transgen, Beijing, China) according to the manufacturer's instructions. The mRNA expression levels of *CRYAB* (XM_021062778.1) and *CTGF* (NM_213833.2) in lung tissues were determined using quantitative real-time PCR (qRT-PCR). There were eight biological replicates for each group (n=8). The primers used to quantify *CRYAB* and *CTGF* were as follows: *CRYAB*-forward, *GAG ATG CGT CTG GAG AAG GA*; *CRYAB*-reverse, *ATC TCC CAA CAC CTT GAC CT* and *CTGF*-forward, *GCT TAC CGA CTG GAA GAC AC*; *CTGF*-reverse, *AGA AAG CGT TGT CAT TGG TA*. Further, *β-actin* (AJ312193) was used as an internal reference gene with the following primers: forward, *TCT GGC ACC ACA CCT TCT A* and reverse, *AAG GTC TCG AAC ATG ATC TG*. qRT-PCR was performed in triplicate using 20μL reaction volumes containing 1.0μL of cDNA, 0.5μL of the respective forward and reverse primers (10.0 nmol/μL), and 10.0μL of 2× SYBR Green Mix (Transgen Ltd., Beijing, China). The procedure for qRT-PCR included an initial denaturation step at 95°C for 30 s, followed by 45 cycles at 95°C for 5 s and at 58°C for 20 s. The mRNA expression levels of the target genes were calculated using the $2^{-\Delta\Delta CT}$ method.

Crude proteins (40μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Subsequently, the proteins were transferred to Immobilon-P transfer membranes using a Bio-Rad Criterion Blotter (Bio-Rad, Hercules, CA, USA). The membranes were blocked overnight in a blocking buffer (Beyotime Ltd., Shanghai, China) and then incubated at 4°C for 2 h with anti-β-tubulin rabbit polyclonal antibody (1:2000 dilution, Abcam, Cambridge, UK), *CRYAB* antibodies (1:2,000 dilution, Abcam), and *CTGF* (1:2,000 dilution, Abcam) antibodies. After washing with phosphate-

buffered saline containing 0.1% Tween 20, the membranes were incubated with secondary horseradish peroxidase-labeled goat anti-rabbit IgG (H + L) (1:5,000 dilution, Abcam) at 37 °C for 1 h. The immune complexes were visualized using an eECL Western Blot Kit (CWBIO Ltd., Beijing, China) according to the manufacturer's instructions. The relative protein levels were determined using *ImageJ v1.51* software (NIH, https://imagej.nih.gov/ij/).

Data regarding the expression levels were analyzed using SPSS v18.0 software (SPSS Inc., Chicago, IL, USA) through one-way analysis of variance to determine statistical significance. Graphs were prepared using SigmaPlot 10.0 (Systat Software, San Jose, CA, USA); data herein are presented as mean ± standard error. A $\chi 2$ test was used to analyze the distribution of genotypes and differences in genotype frequencies.

## RESULTS

After screening the 5′ flanking region of *CRYAB* and *CTGF*, four SNPs were identified. Two SNPs, g.39644482C>T and g.39644132T>C, were located upstream of the initiation codon of *CRYAB* (Fig. 1). Similarly, two other SNPs, g.31671748A>G and g.31671773T>G, were located upstream of the initiation codon of *CTGF* (Fig. 1). The genotype and allele frequencies of the four SNPs are shown in Table 2. The distributions of the genotypes of the four SNPs in the TP and YY populations conformed to the Hardy–Weinberg equilibrium. Differences in the genotype frequencies were statistically significant for g.39644482C>T and g.39644132T>C (*CRYAB*, $P < 0.01$), as well as for g.31671748A>G and g.31671773T>G (*CTGF*, $P < 0.01$). Evaluation of the predicted transcription factor binding sites revealed that the probable transcription factor FOXP2 could be replaced by GATA4 at the g.39644482C>T locus and the potential transcription factor RXRA could be substituted by FOXH1 at the g.39644132T>C locus (Table 2). At the g.31671748A>G and g.31671773T>G loci, the potential transcription factors MAFK and PRDM1 could similarly be replaced by STAT5 and FOSL2, respectively, as a result of the SNPs (Table 2).

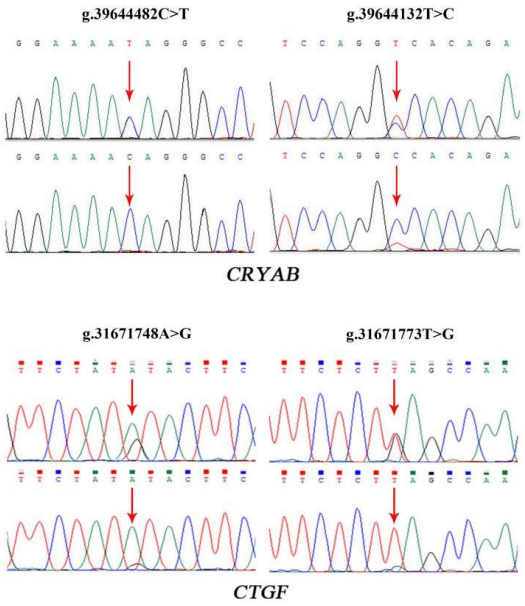


Figure 1. Identification of SNPs located at the 5′ flanking region of *CRYAB* and *CTGF* genes.

Upon investigating the mRNA expression of *CRYAB* and *CTGF* in the lung tissues of TPs and YYs via qRT-PCR (Fig. 2), it was revealed that the expression levels of *CRYAB* mRNA increased significantly in TPs with an increase in altitude ($P<0.05$ or $P<0.01$). However, in YYs, the expression level of *CRYAB* mRNA was higher at 2900 m than at 100 and 3700 m ($P<0.05$ and $P<0.01$, respectively). At 2900 m, the *CRYAB* mRNA level was significantly higher in YYs than in TPs ($P<0.05$). For *CTGF*, the mRNA expression levels increased in TPs as the altitude increased; the opposite was observed in YYs (i.e., the *CTGF* mRNA levels decreased as the altitude increased). The *CTGF* mRNA level was significantly higher in the YYs than in the TPs at 100 m, whereas it was significantly lower in the YYs than in the TPs at 3700m.

Table 2. Genotype and allele frequencies of four SNPs of *CRYAB* and *CTGF* genes in two pig breeds

| | Loci | Breed | Genotype frequency（Number/Frequency） | | | $X^2$ value (*p* value) | Allele gene frequency | | Predicated transcription factor binding |
|---|---|---|---|---|---|---|---|---|---|
| | | | CC | CT | TT | | C | T | |
| *CRYAB* | g.39644482C>T | YY | 45/1 | 0/0 | 0/0 | 9.014 (0.006) | 1 | 0 | *FOXP2—GATA4* |
| | | TP | 37/0.673 | 16/0.291 | 2/0.036 | 3.112 (0.207) | 0.818 | 0.182 | |
| | | TP vs YY Fisher's Exact Test=20.386 *P*=6.94E-6 | | | | | | | |
| | | | TT | CT | CC | | C | T | |
| | g.39644132T>C | YY | 45/1 | 0/0 | 0/0 | 14.567 (2.10E-4) | 0 | 1 | *RXRA—FOXH1* |
| | | TP | 28/0.509 | 22/0.400 | 5/0.091 | 5.559 (0.062) | 0.291 | 0.709 | |
| | | TP vs YY Fisher's Exact Test=34.797 *P*=4.99E-9 | | | | | | | |
| | | | AA | AG | GG | | A | G | |
| | g.31671748A>G | YY | 26/0.867 | 4/0.133 | 0/0 | 8.156 (0.013) | 0.933 | 0.067 | *MAFK—STAT5* |
| | | TP | 15/0.326 | 24/0.522 | 7/0.152 | 4.556 (0.102) | 0.587 | 0.413 | |
| | | TP vs YY Fisher's Exact Test=22.147 *P*=6.62E-6 | | | | | | | |
| *CTGF* | | | TT | TG | GG | | T | G | |
| | g.31671773T>G | YY | 30/1 | 0/0 | 0/0 | 26.635 (6.19E-7) | 1 | 0 | *PRDM1—FOSL2* |
| | | TP | 2/0.043 | 3/0.065 | 41/0.891 | 19.047 (3.42E-5) | 0.076 | 0.924 | |
| | | TP vs YY Fisher's Exact Test=80.048 *P*=3.84E-19 | | | | | | | |

The protein expression levels of CRYAB and CTGF were also investigated. Bands of CRYAB, CTGF, and β-tubulin (as an internal control) were visualized at 22, 38, and 55 kDa, respectively. A similar expression pattern was observed in the protein and mRNA of *CRYAB*, except in YYs at 2900 m (Fig. 3). There was no significant difference in CRYAB between YYs at 2900 and 3700 m. The same expression pattern was observed between the protein and mRNA of *CTGF*. No significant difference in CTGF was observed between TP at 2900 and 3700 m, whereas significant differences were identified at the mRNA level. Overall, with increasing altitude, CTGF increased in TP and decreased in YY.

## DISCUSSION

Our previous study demonstrated that *CRYAB* expression was significantly higher in the heart tissue of highland pigs than in lowland pigs and in highland TPs than in highland YYs but lower in lowland TPs than in lowland YYs (Zhang *et al*., 2017). In this study, based on mRNA and protein levels, *CRYAB* was also in the lung tissue of pigs in highland regions, i.e., 2900 and 3700m above mean sea level, pigs in the lowland region (Fig. 2 and Fig. 3). This is consistent with the expression pattern of CRYAB in heart tissue. *CRYAB*, also referred to as *HspB5* or αB-crystallin, is a member of the small heat shock protein family; it plays an important role in blocking the aggregation of denatured proteins and is expressed in the heart and lungs (Parcellier *et al*., 2005; Xu *et al*., 2008; Harakalova *et al*., 2015; Haslbeck and Vierling, 2015; Rajagopal *et al*., 2015). As an anti-apoptotic molecular chaperone protein, it can maintain the correct balance between intracellular protein synthesis and degradation and is involved in the progression of many pathological processes, including misfolding of proteins caused by stress as well as by changes in pH and temperature in the environment (Hartl *et al*., 2011; Tarone and Brancaccio, 2014). *CRYAB* was found to be downregulated in a mouse model of pulmonary arterial hypertension induced by chronic hypoxia (Clumas *et al*., 2013). Conversely, in this study, hypoxia induced *CRYAB* mRNA and protein expression. Interestingly, the mRNA level of *CRYAB* was lower in the lung tissue of TPs than in that of YYs at 2900-m above mean sea level (Fig. 2). This implies that TPs could adapt better to the hypoxic environment than YYs.

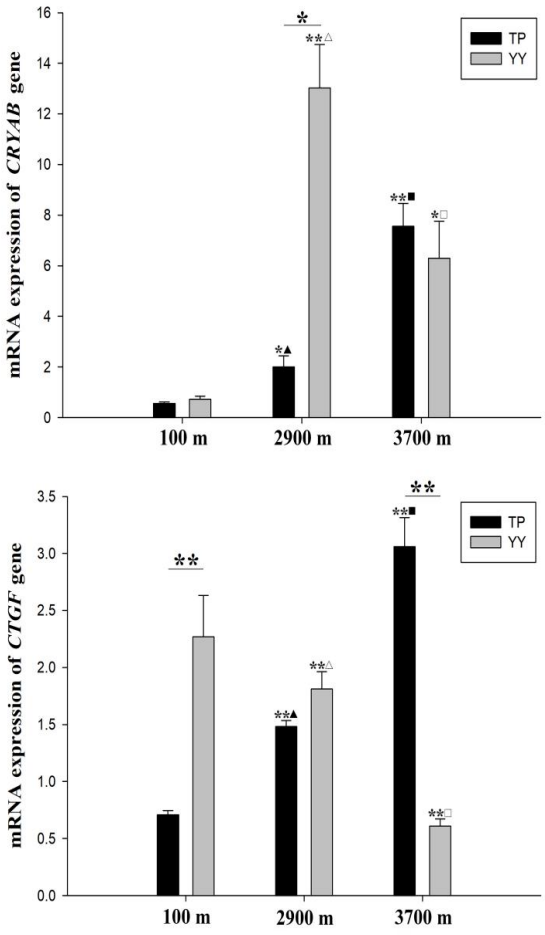


Figure 2. Relative mRNA expression level of *CRYAB* and *CTGF* in two pig breeds at different altitudes.

*represents a significant difference ($P < 0.05$), ** represents an extremely significant difference ($P < 0.01$). ▲/△represents the comparison of the same breed between altitudes of 2900 and 100 m, ■/□represents the comparison of the same breed between altitudes of 3700 and 2900 m. TP, Tibetan pig; YY, Yorkshire pig.

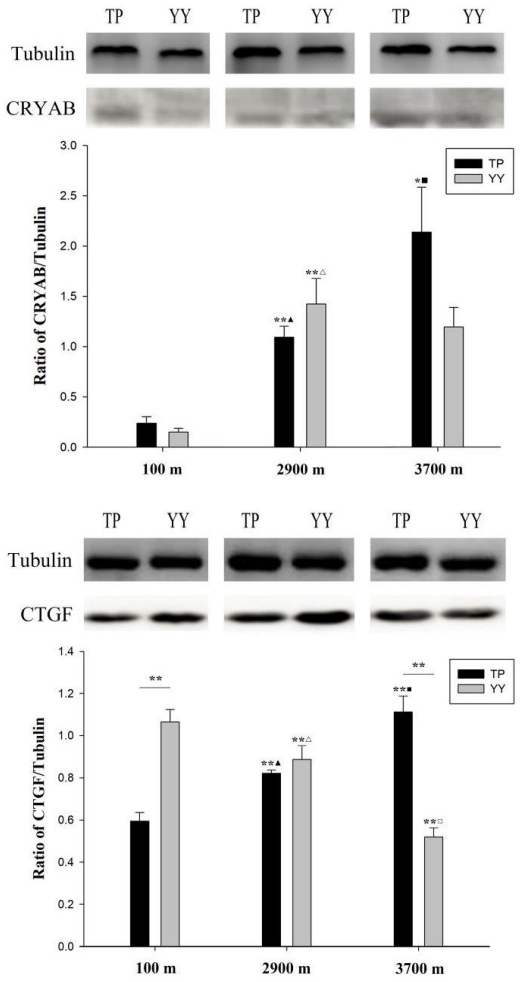


Figure 3. CRYAB and CTGF levels in the lung tissues of two pig breeds at different altitudes

*represents a significant difference ($P < 0.05$), ** represents an extremely significant difference ($P < 0.01$). ▲/△represents the comparison of the same breed between altitudes of 2900 and 100 m, ■/□represents the comparison of the same breed between altitudes of 3700 and 2900 m. TP, Tibetan pig; YY, Yorkshire pig.

Our previous study also revealed that the mRNA level of *CTGF* was significantly higher in the heart tissue of TPs than in that of YYs, in lowland as well as highland conditions (Zhang *et al*., 2017). Further, the *CTGF* mRNA level was higher in the heart tissue of highland TPs than in that of lowland TPs, but there was no significant difference among YYs in this regard (Zhang *et al*., 2017). In this study, however, the *CTGF* mRNA level in the lung tissue of TPs increased but that in the lung tissue of YYs decreased as the altitude increased (Fig. 2 and Fig. 3). *CTGF*, also referred to as *CCN2*, is a member of the CCN (immediate early gene) family, which plays an important role in collagen production and lung development (Riser *et al*., 2010; Riser *et al*., 2015). It has been demonstrated that hypoxia time-dependently increased *CTGF* expression in both human lung fibroblast cell line and primary human lung fibroblasts (Cheng *et al*., 2016). *CTGF* is known to exacerbate vascular remodeling in the lungs (Pi *et al*., 2018). It has also been reported that *CTGF* deletion could protect against the development of pulmonary hypertension secondary to chronic hypoxia (Gomez *et al*., 2020). Combining the function of CTGF in vascular remodeling and

expression pattern in lung and heart tissue under hypoxia, we speculated that the function of vascular remodeling was destroyed in YY caused by hypoxia condition, whereas hypoxia treatment did not significantly affect the vascular structure in YY heart. TPs, originating from the Tibetan Plateau, have undergone long-term natural selection in a hypoxic environment (Shang *et al*., 2019). Thus, compared with lowland YYs, TPs should possess adaptations to hypoxia. This might help us decipher the *CTGF* expression pattern under hypoxia in pig lungs and thus deserves further exploration.

The difference in expression levels could also be partially explained by genetic differences, such as the binding of different transcriptional factors. In this study, two novel SNPs were identified within 2000-bp upstream of the *CRYAB* and *CTGF* initiating codons. Significant allelic and genotypic differences were observed between TPs and YYs for these four SNPs. Mutations at these four loci were found to be associated with changes in predicted transcription factor binding sites. As a result, for the g.39644482C>T site, the transcriptional factor FOXP2 was substituted by GATA4 (Tab. 2). GATA4, a member of the GATA family, specifically binds to the structure of (T/A)GATA(A/G), which affects cell proliferation, angiogenesis, and cardiopulmonary development (He *et al*., 2007). The absence of GATA4 induces endothelial cell differentiation suppression, lung malformations, and distal airway dilatation in mice (Cao *et al*., 2020). Similarly, the C allele at g.39644132T>C results in the preferential binding by the transcription factor FOXH1 as opposed to RXRA (Tab. 2). The inactivation of FOXH1 is reported to be associated with lung development, which includes the transformation of the one-lobed left lung into the four-lobed right lung (Hoodless *et al*., 2001; von Both *et al*., 2004). For the g.31671748A>G site, the transcription factor was changed from MAFK to STAT5 (Tab. 2). STAT5, a member of the STAT family, promotes cell cycle progression, proliferation, invasion, and angiogenesis and inhibits apoptosis (Pastuszak-Lewandoska *et al*., 2014). The G allele at g.31671773T>G leads to the preferential binding of the transcription factor FOSL2 rather than PRDM1 (Tab. 2). The GG genotype is dominant in TPs, whereas TT is dominant in YYs. FOSL2, a member of the c-Fos transcription factor family, regulates TGF-β1 signaling in non-small cell lung cancer, and its inactivation reduces cell viability in hypoxia (Wang *et al*., 2014; Vancauwenberghe *et al*., 2019).

In conclusion, the expression patterns of *CRYAB* and *CTGF* in highland, middle-highland, and lowland TPs and YYs were clarified in this study. These differences in expression patterns at different altitudes might contribute to their hypoxia adaptation. Furthermore, four novel SNPs (g.39644482C>T and g.39644132T>C in *CRYAB*; g.31671748A>G and g.31671773T>G in *CTGF*) were identified within 2000-bp upstream of the *CRYAB* and *CTGF* initiating codons. Potential transcriptional factors of the four SNPs were identified. To some extent, changing of transcriptional factors may partially contribute to the expression differences observed between the TPs and YYs. These findings provide novel insights into the high-altitude hypoxia adaptations of TPs. We believe that our analysis of the specific expression patterns and genetic characteristics of *CRYAB* and *CTGF* genes in TPs will help reveal the mechanisms by which organisms adapt to low-oxygen environments, with an aim to contribute to the study of species evolution and human diseases.

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## AUTHOR CONTRIBUTIONS

Study conception and design: PS. Experimentation and data analysis: JZ, SYL, LX, XYG, MBW. Contribution toward reagents/materials/analysis tools: JZ, KJW, MQD, BZ, ZMW. Writing and revising of manuscript: PS, HZ, SYL, and LX.

## REFERENCES


ALLEN, J.T.; SPITERI, M.A. Growth factors in idiopathic pulmonary fibrosis: relative roles. *Respir. Res*., v.3, p.13-21, 2002.

BELLAYE, P.S.; WETTSTEIN, G.; BURGY, O. *et al*. The small heat-shock protein αB-crystallin is essential for the nuclear localization of Smad4: impact on pulmonary fibrosis. *J. Pathol*., v.232, p.458-472, 2014.

CAO, Y.; GUO, J.; ZHANG, J.; LI, L. *et al*. HYDIN loss-of-function inhibits GATA4 expression and enhances atrial septal defect risk. *Mech. Dev*., v.162, p.103611, 2020.

CHEN, H.Y.; LIN, C.H.; CHEN, B.C. ADAM17/EGFR-dependent ERK activation mediates thrombin-induced CTGF expression in human lung fibroblasts. *Exp. Cell Res*., v.370, p.39-45, 2018.

CHENG, Y.; LIN, C.H.; CHEN, J.Y. *et al*. Induction of connective tissue growth factor expression by hypoxia in human lung fibroblasts via the MEKK1/MEK1/ERK1/GLI-1/GLI-2 and AP-1 pathways. *PLoS ONE*., v.11, e0160593, 2016.

CHRISTOPHER, K.L.; PEDLER, M.G.; SHIEH, B. *et al*. Alpha-crystallin-mediated protection of lens cells against heat and oxidative stress induced cell death. *Biochim. Biophys. Acta*, v.1843, p.309-315, 2014.

CLUMAS, M.; EYRIES, M.; POIRIER, O. *et al*. Bone morphogenetic proteins protect pulmonary microvascular endothelial cells from apoptosis by upregulating α-B-crystallin. *Arterioscler. Thromb. Vasc. Biol*., v.33, p.2577-2584, 2013.

GOMEZ, A.P.; MORENO, M.J.; HERNÁNDEZ, A. *et al*. Adventitial growth and lung connective tissue growth factor expression in pulmonary arterioles due to hypobaric hypoxia in broilers. *Poult. Sci*., v.99, p.1832-1837, 2020.

HARAKALOVA, M.; KUMMELING, G.; SAMMANI, A. *et al*. A systematic analysis of genetic dilated cardiomyopathy reveals numerous ubiquitously expressed and muscle-specific genes. *Eur. J. Heart Fail*., v.17, p.484-493, 2015.

HARTL, F.U.; BRACHER, A.; HAYER-HARTL, M. *et al*. Molecular chaperones in protein folding and proteostasis. *Nature*, v.475, p.324-332, 2011.

HASLBECK, M.; VIERLING, E. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. *J. Mol. Biol*., v.427, p.1537-1548, 2015.

HE, C.; CHENG, H.H.; ZHOU, R.J. GATA family of transcription factors of vertebrates: phylogenetics and chromosomal synteny. *J. Biosci*., v.32, p.1273-1280, 2007.

HOODLESS, P.A.; PYE, M.; CHAZAUD, C. *et al*. FoxH1 (Fast) functions to specify the anterior primitive streak in the mouse. *Genes Dev*., v.15, p.1257-1271, 2001.

KOSMIDER, B.; MESSIER, E.M.; CHU, H.W. *et al*. Human alveolar epithelial cell injury induced by cigarette smoke. *PLoS One*, v.6, e26059, 2011.

LIU, R.; JIN, L.; LONG, K. *et al*. Analysis of mitochondrial DNA sequence and copy number variation across five high-altitude species and their low-altitude relatives. *Mitochondrial DNA B Resour.*, v.3, p.847-851, 2018.

MITRA, A.; BASAK, T.; DATTA, K. *et al*. Role of α-crystallin B as a regulatory switch in modulating cardiomyocyte apoptosis by mitochondria or endoplasmic reticulum during cardiac hypertrophy and myocardial infarction. *Cell Death Dis*., v.4, p.114-125, 2013.

PARCELLIER, A.; SCHMITT, E.; BRUNET, M. *et al*. Small heat shock proteins HSP27 and alphaB-crystallin: cytoprotective and oncogenic functions. *Antioxid. Redox Signal.*, v.7, p.404-413, 2005.

PASTUSZAK-LEWANDOSKA, D.; DOMAŃSKA, D.; CZARNECKA, K.H. *et al*. Expression of STAT5, COX-2 and PIAS3 in correlation with NSCLC histhopathological features. *PLoS One*, v.9, e104265, 2014.

PI, L.; FU, C.; LU, Y. *et al*. Vascular endothelial cell-specific connective tissue growth factor (CTGF) is necessary for development of chronic hypoxia-induced pulmonary hypertension. *Front. Physiol*., v.9, p.138-150, 2018.

RAJAGOPAL, P.; TSE, E.; BORST, A.J. *et al*. A conserved histidine modulates HSPB5 structure to trigger chaperone activity in response to stress-related acidosis. *Elife*, v.4, e07304, 2015.

REDDY, V.S.; REDDY, G.B. Role of crystallins in diabetic complications. *Biochim. Biophys. Acta*, v.1860, p.269-277, 2016.

RISER, B.L.; BARNES, J.L.; VARANI, J. Balanced regulation of the CCN family of matricellular proteins: a novel approach to the prevention and treatment of fibrosis and cancer. *J. Cell Commun. Signal*., v.9, p.327-339, 2015.

RISER, B.L.; NAJMABADI, F.; PERBAL, B. *et al*. CCN3/CCN2 regulation and the fibrosis of diabetic renal disease. *J. Cell Commun. Signal.*, v.4, p.39-50, 2010.

SAMBROOK, J.; D. FUSSELL. *Molecular cloning*: a laboratory manual. 3.ed. New York: Cold Spring Harbor Laboratory Press, 2001. p.112-120.

SHANG, P.; LI, W.; LIU, G. *et al*. Identification of lncRNAs and genes responsible for fatness and fatty acid composition traits between the Tibetan and Yorkshire pigs. *Int. J. Genomics*, v.1, p.5070975, 2019.

TANG, M.; CHEN, B.; LIN, T. *et al*. Restraint of angiogenesis by zinc finger transcription factor CTCF-dependent chromatin insulation. *Proc. Natl. Acad. Sci. USA*, v.108, p.15231-15236, 2011.

TARONE, G.; BRANCACCIO, M. Keep your heart in shape: molecular chaperone networks for treating heart disease. *Cardiovasc. Res*., v.102, p.346-361, 2014.

UENO, M.; MAENO, T.; NOMURA, M. *et al*. Hypoxia-inducible factor-1alpha mediates TGF-beta-induced PAI-1 production in alveolar macrophages in pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol*., v.300, p.L740-752, 2011.

VANCAUWENBERGHE, E.; BOLLAND, H.; CARROLL, C. *et al*. Defining the role of AP1 in molecular adaptation to hypoxia in colorectal cancer. *Cancer Res*., v.79, p.2652, 2019.

VON BOTH, I.; SILVESTRI, C.; ERDEMIR, T. *et al*. Foxh1 is essential for development of the anterior heart field. *Dev. Cell.*, v.7, p.331-345, 2004.

WANG, J.; SUN, D.; WANG, Y. *et al*. FOSL2 positively regulates TGF-β1 signaling in non-small cell lung cancer. *PLoS One*, v.9, e112150, 2014.

XU, Q.Y.; GAO, Y.; LIU, Y. *et al*. Identification of differential gene expression profiles of radioresistant lung cancer cell line established by fractionated ionizing radiation in vitro. *Chin. Med. J.*, v.121, p.1830-1837, 2008.

ZHANG, B.; CHAMBA, Y.; SHANG, P. *et al*. Comparative transcriptomic and proteomic analyses provide insights into the key genes involved in high-altitude adaptation in the Tibetan pig. *Sci. Reprod*., v.7, p.3654-3665, 2017.