

CASE REPORT

Open Access



Diagnosis of Klippel-Trenaunay syndrome and extensive heterotopic ossification in a patient with a femoral fracture: a case report and literature review

Wanbo Zhu^{1,2†}, Kai Xie^{2†}, Jiazhao Yang^{2†}, Li Li², Xujin Wang², Lei Xu^{2*} and Shiyuan Fang^{1,2*}

Abstract

Background: Klippel-Trenaunay syndrome (KTS) is a rare complex vessel malformation syndrome characterized by venous varicosities, capillary malformations, and limb hypertrophy. However, extensive heterotopic ossification (HO) secondary to this syndrome is extremely rare.

Case presentation: We report the case of a patient with previously undiagnosed KTS and extensive HO who presented with a femoral fracture secondary to a motor vehicle accident. Extensive ossification, which leads to compulsive contracture deformity and dysfunction of the leg, was distributed on the flexor muscle side, as revealed by the radiograph. The diagnosis was finally established by combining imaging and histological analysis with classical clinical symptoms. Amputation was performed at the fracture site proximal to the infected necrotic foci. Open management of the fracture was challenging owing to the pervasive ossification and tendency for excessive bleeding. Gene sequencing analysis showed homozygous mutation of FoxO1 gene.

Conclusions: Definitive diagnosis of a combination of KTS and extensive HO requires detailed imaging analysis and pathologic evidence. Mutation of the FoxO1 gene, which regulates bone formation by resistance to oxidative stress in osteoblasts, is a potential factor in the microenvironment of malformed vessels caused by KTS.

Keywords: Klippel-Trenaunay syndrome, Heterotopic ossification, FoxO1

Background

Klippel-Trenaunay Syndrome (KTS) is a rare congenital disorder of the vascular system that is usually diagnosed at birth. It mostly occurs in one limb in the lower extremities and is characterized by a clinical triad of a) capillary malformations (port wine stain); b) varicose veins

or venous malformations; and c) soft tissue and bone hypertrophy [1]. Clinical diagnosis requires the presence of at least two of these signs. When arteriovenous malformations coexist, Klippel-Trenaunay-Weber syndrome (or Parkes-Weber) is diagnosed [2]. Fracture of the long bones in KTS requires careful management owing to profuse bleeding, non-healing wounds, infection, and non-union of the bone [3, 4].

Heterotopic ossification (HO) is pathological bone formation at extra-skeletal sites, such as soft tissues, which limits bone and joint activity [5]. HO is often divided into the acquired non-genetic form and

* Correspondence: bayinhexl@126.com; fangshiyuan2008@126.com

[†]Wanbo Zhu, Kai Xie and Jiazhao Yang contributed equally to this work.

²Department of Orthopedics, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230001, Anhui, China

¹Affiliated Anhui Provincial Hospital of Anhui Medical University, Hefei 230001, Anhui, China



inherited genetic form. Extensive HO is often caused by severe trauma or due to inherited conditions, such as fibrodysplasia ossificans progressiva or progressive osseous heteroplasia [6]. However, to the best of our knowledge, there are no published reports of HO combined with KTS or HO secondary to KTS.

We describe a rare case of subtrochanteric femoral fracture in a patient with KTS and extensive HO in the leg.

Case presentation

A 46-year-old man was transferred to our emergency department with a 15-cm stitched wound on a large

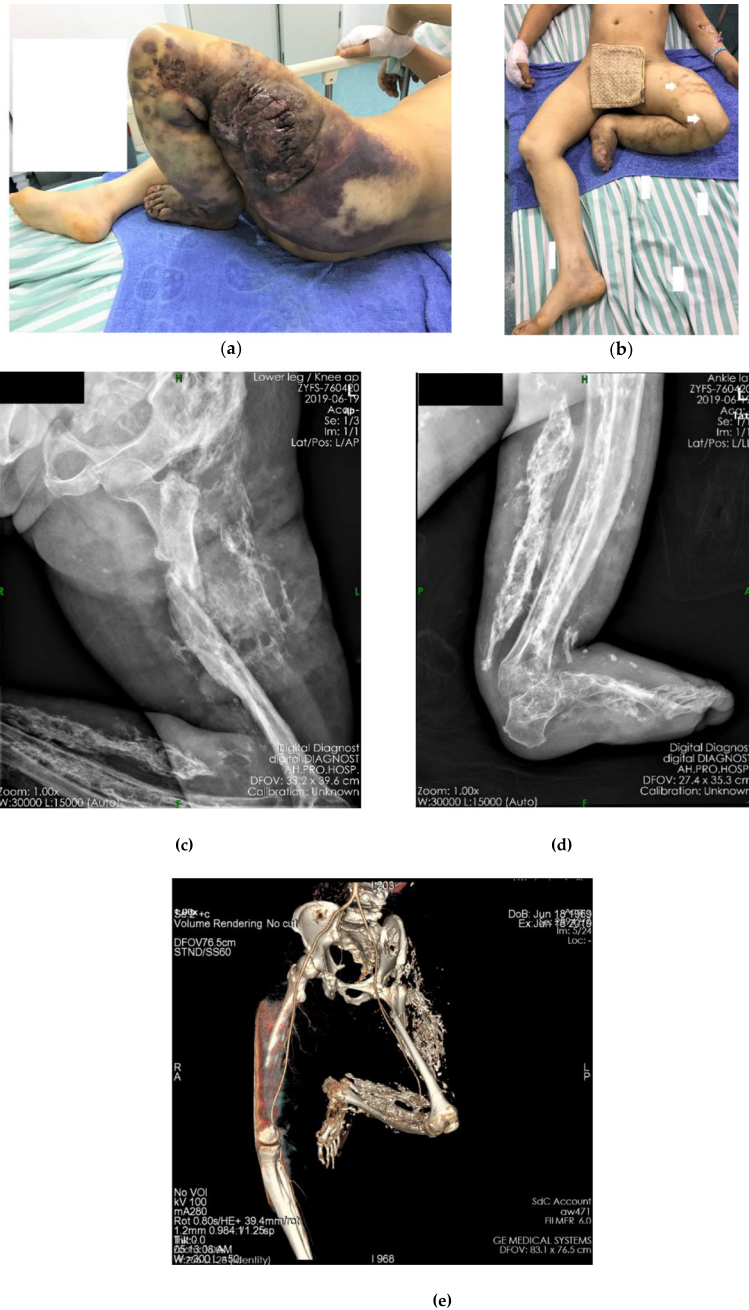


Fig. 1 Photographs and images of the injury. **a** Initial presentation of hemangioma, a 15-cm stitched wound can be seen on the hemangioma; **b** Contracture of the leg, the white arrows show superficial varicose veins; **c-d** Radiograph showing femoral fracture and extensive high-density shadows distributed at the flexor muscle side of left lower extremity; **e** Three-dimensional computed tomography (3DCT). Vascular reconstruction shows coherence and integrity of the main arteries of the lower extremities

kermesinus hemangioma-like lump on the left lateral thigh (Fig. 1a). The patient was hit by a car 1 day before. The patient was admitted immediately. The entire left lower extremity presented a compulsive contracture deformity with massive swelling, induration, and varicosities (Fig. 1b). Systemic examination revealed severe anemia, and an emergency transfusion was performed. Radiographs of the left leg showed a shaft fracture at the proximal third of the femur with extensive high-density shadows distributed in the flexor muscle side (Fig. 1c-d). Ultrasonography of the limb showed normal blood flow of the main vessels and venous malformations in the dermis. Three-dimensional computed tomography (CT) reconstruction confirmed a femoral fracture and a continuous artery with massive skeletal structural deformities along the extremity (Fig. 1e). The contralateral limb radiograph revealed a normal skeletal structure.

The patient claimed to have a “port-wine birthmark” on the left foot at birth. With growth and development, the port-wine stain started to spread, and the entire left lower limb was progressively flexed and contracted with

loss of function. The condition stabilized after his growth stopped. Magnetic resonance imaging (MRI) suggested diffuse soft tissue hemangiomas, and a diagnosis of KTS was made based on the medical history and clinical characteristics (Fig. 2a and b). A technetium 99 m-methyl diphosphonate (99mTc-MDP) bone scan also indicated extensive radioactivity concentration on the flexor side of the limb, and a diagnosis of HO was inferred (Fig. 2c). However, it was difficult to distinguish whether the high-density shadow and radioactive concentration exhibited on imaging was intramuscular calcification or ossification.

After hospitalization, the patient’s lateral thigh wound began to show signs of necrosis on the 3rd day. Lower extremity digital subtraction angiography (DSA) showed vascular distribution and blood supply of the diseased limb (Fig. 3a-b). No arteriovenous fistulas were found during DSA. On the fifth day, bacterial culture from the wound showed a gram-positive bacterial infection. On the seventh day, an amputation was performed at the fracture site to the proximal of the infected necrotic foci.

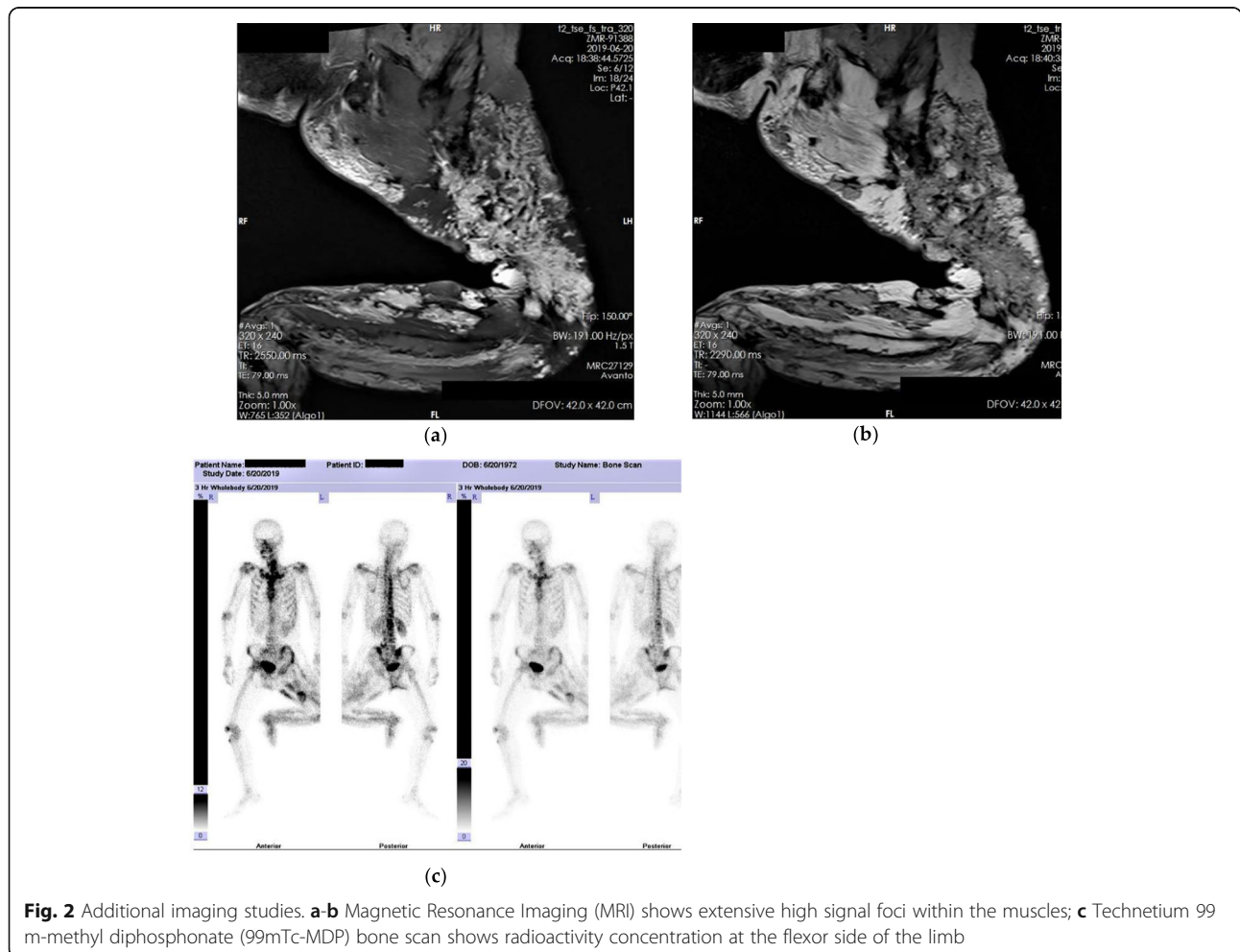


Fig. 2 Additional imaging studies. **a-b** Magnetic Resonance Imaging (MRI) shows extensive high signal foci within the muscles; **c** Technetium 99 m-methyl diphosphonate (99mTc-MDP) bone scan shows radioactivity concentration at the flexor side of the limb

Owing to the high amputation plane, a tourniquet could not be used. Due to extensive vascular malformations and soft tissue ossification, bleeding during surgery was excessive and difficult to control by conventional electrocoagulation and ligation. Extensive ossification impeded the progress of the surgery. The intraoperative blood transfusion was 17 units. The patient was transferred to the intensive care unit (ICU) for advanced life support. Ossification specimens provided histopathologic evidence of HO (Fig. 3c-d). Normal trabecular bone formation and bone structure construction were detected. The patient was discharged on the 33rd day after hospitalization, and a postoperative X-ray was performed (Fig. 4). In order to maintain hemoglobin stability, the patient was

transfused 55 units of blood during hospitalization. On follow-up at 2 months, the amputation wound healed well.

The patient and his family provided informed consent for genetic testing. Gene sequencing was performed using next-generation sequencing technology (NGS). Heterozygous mutations at chromosome 13 in both parents led to a homozygotic mutation in the patient, resulting in a FoxO1 translation error (Fig. 5a-b). The sequencing results were explained to the subjects according to the American College of Medical Genetics and Genomics guidelines [7]. The study was approved by the local ethics committee.

The NGS sequencing of the FoxO1 gene revealed a novel homozygotic missense variation in Exon 2,

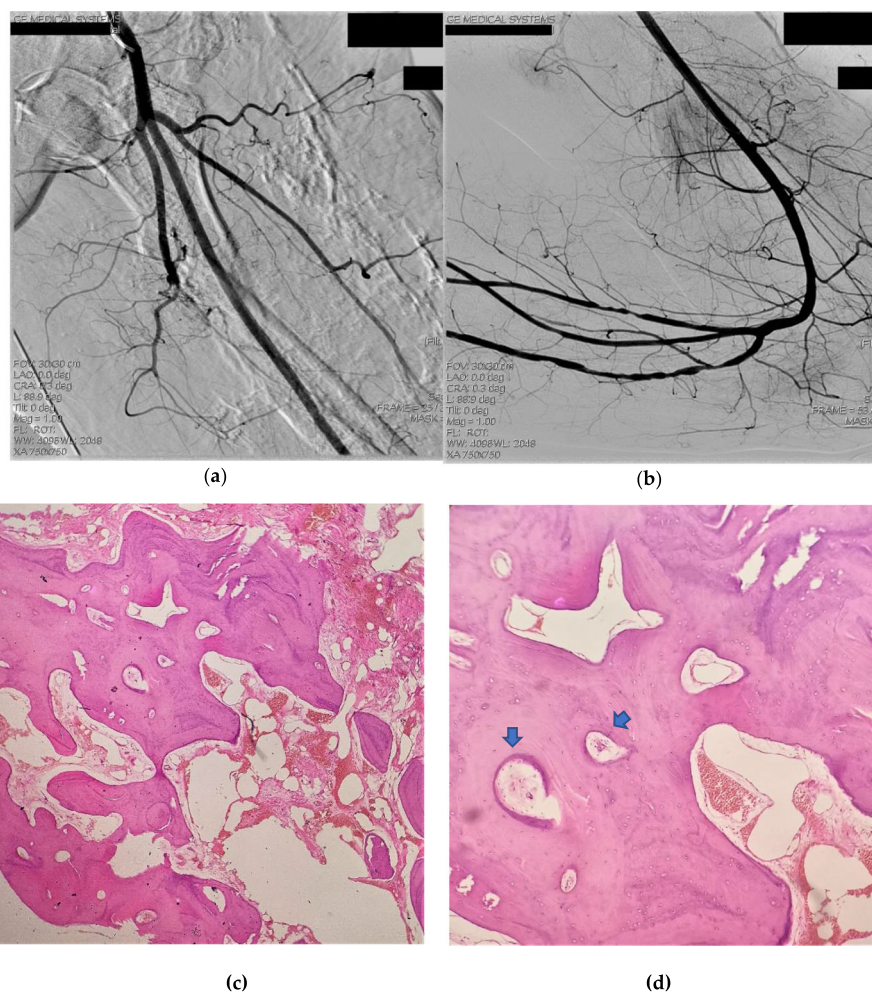


Fig. 3 Necrosis and surgical outcome. **a-b** Lower extremity digital subtraction angiography shows vascular distribution. **c-d** Pathological specimens of ossification shows trabecular bone formation and bone structure construction. **c** magnification $\times 100$ and **d** magnification $\times 40$ (Hematoxylin & Eosin staining). The blue arrows show osteocytes



Fig. 4 Postoperative X-ray showed fracture site after amputation

c.1532C > T, leading to a translation error at amino acid 551 (p.A551V). It was predicted to be disease causing based on Sorting Intolerant From Tolerant (SIFT) analysis. The SIFT score was 0.041, and the SIFT converted rank score was 0.419.

Discussion and conclusions

In this study, we report the case of a patient with a femoral fracture secondary to a motor vehicle accident diagnosed with KTS following imaging and histological analysis.

Mutation of the FoxO1 gene, which regulates bone formation by resistance to oxidative stress in osteoblasts, is a potential factor in the microenvironment of malformed vessels caused by KTS.

Vascular malformations in KTS usually affect the capillary, venous, and lymphatic systems of the lower extremities, which leads to swelling, varices, and ulcerations of the diseased limb [1]. Elevated D-dimer levels and mutation of the AGGF1 gene are considered to suggest the diagnosis [8]. However, owing to coagulation disorders caused by the fracture in our case, D-dimer was high during hospitalization and could not be considered suggestive of KTS [9]. Whole genome sequencing did not reveal AGGF1 or PIK3CA mutations. MRI is essential for the diagnostic evaluation of KTS as it reveals differences in the vascular malformations and soft tissues [10]. Multiple high signal foci within the muscles can be seen on T2 SE sequences. In addition, duplex ultrasound imaging and DSA provide indirect and direct evidence of vascular morphology and function, providing references for diagnosis.

Clinical diagnosis of KTS depends on the classic presentation [11]. Clinical presentation of KTS in our case was typical; however, flexion deformity of the lower extremities is not common. We found a case of KTS with lower extremity contracture, but no ossification was found on MRI and CT [10]. The author attributed the contracture to muscle atrophy and disuse. In this case, the presence of extensive ossification of the lower extremity flexor may have caused lower extremity contracture. Diagnosis of HO mostly depends on radiographic imaging and clinical history. CT, single photon emission CT (SPECT), and bone scanning may help to identify the extent of ossification and aid in early detection [12]. However, the gold standard for HO diagnosis is still the

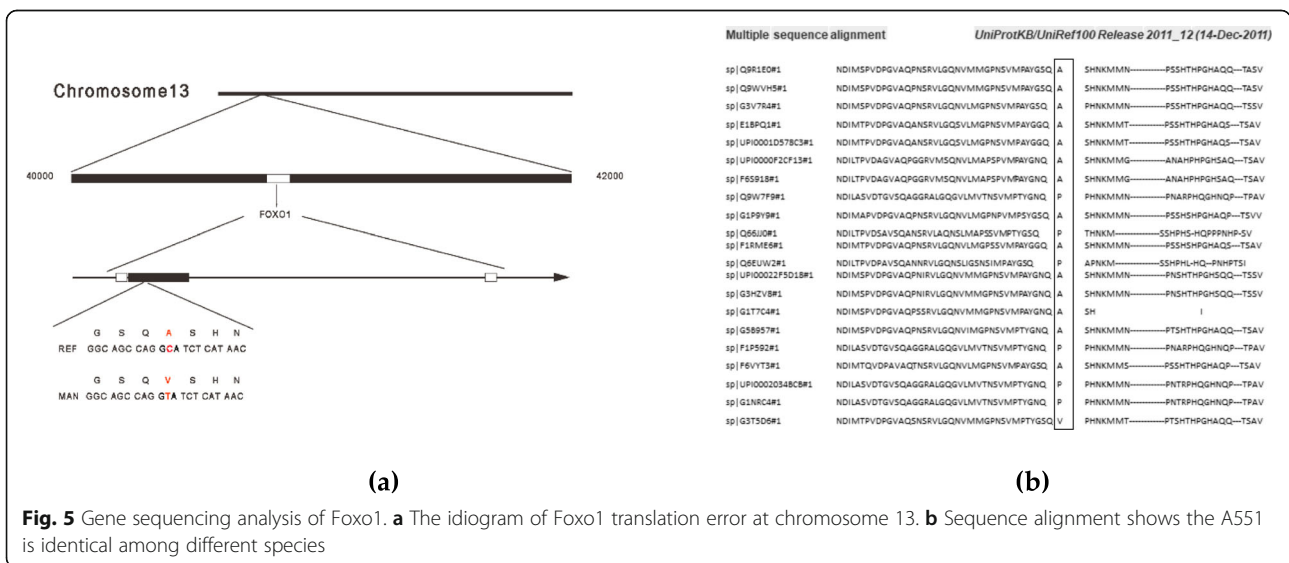


Fig. 5 Gene sequencing analysis of Foxo1. **a** The idiomogram of Foxo1 translation error at chromosome 13. **b** Sequence alignment shows the A551 is identical among different species

pathological results of tissue biopsy suggesting bone trabecular growth and bone structure formation [13].

No inherited HO-related gene mutation was found using whole exome sequencing. No history of traumatic head or spinal cord injury was claimed. No possible pathogenic lesion was detected on brain or spinal MRI. The combination of clinical symptoms and history, suggested the diagnosis of acquired HO. Acquired HO refers to abnormal bone tissue outside the normal skeletal system. Uncontrolled signal transduction plays a key role in recruiting and inducing the differentiation of progenitor cells such as mesenchymal stem cell and mesenchymal progenitor cell, promoting bone formation and remodeling [5]. Acquired HO is mostly induced by orthopedic trauma or neurogenic injuries [14]. Hypoxia and inflammation are associated with the episodic induction of HO [15]. Hypoxia-inducible factor-1 α inhibits fusion of inclusion body regulated by rabaptin5, thereby, regulating intracellular BMP receptor activity and activating BMP signaling pathway [16]. The formation of HO can be induced through both BMP/Smad pathway and BMP/P38 MAPK pathway [17–19]. In addition, signaling pathway including Hedgehog, Wnt- β -catenin and NF- κ B also contribute to formation of HO [20–22]. In our case, a local hypoxic environment caused by the extensive hemangioma and vascular malformation is a possible mechanism for inducing ossification [23]. The inflammatory response caused by small trauma in the capillary network is also a potential predisposing factor. Although KTS belongs to the PIK3CA-related overgrowth spectrum, we did not find mutations in the PIK3CA gene [24]. In addition, to the best of our knowledge, such extensive ossification has not been seen in previous reports on KTS, so the cause of this series of reactions is worth analyzing.

FoxO1 is a crucial regulator of osteoblast physiology as it is required for proliferation and redox balance in osteoblasts and thereby controls bone formation [25]. Recent research found that FoxO1 provides a favorable intracellular environment for osteoblast functions by defending against the adverse effects of oxidative stress [26]. We utilized NGS analysis to detect a novel homozygous missense variation at FoxO1 in this patient. We hypothesized that due to mutations in FoxO1, the process of favoring protein synthesis and resistance to oxidative stress in osteoblasts was enhanced, promoting bone formation and ossification in the microenvironment of extensive malformed capillaries. In recent studies, it has been confirmed that the expression of FoxO1 is associated with multiple osteogenic phenotypic markers like Runx2 and BMP2, which play an important role in the regulation of osteogenesis [27]. The mechanism of acquired ossification regulated by FoxO1 still requires further studies.

To the best of our knowledge, this is the first reported case of a patient with both KTS and extensive HO, which led to severe lower extremity contracture. This case demonstrates that definitive diagnosis of a combination of these two rare diseases requires detailed imaging analysis and pathologic evidence. Further, it illustrates the challenges of open operation for fractures in KTS patients and the need to anticipate excessive bleeding. Mutations of FoxO1 are the potential regulator of the acquired ossification with KTS, and understanding the exact mechanism requires further research.

Abbreviations

CT: computed tomographic; FoxO: forkhead box O (FoxO) transcription factors; HO: heterotopic ossification; KTS: Klippel-Trenaunay Syndrome; MRI: Magnetic Resonance Imaging

Acknowledgements

The authors thank all the other staff of the traumatic orthopedics department of Anhui emergency center of the First Affiliated Hospital of USTC for their support.

Author contributions

WZ and XK drafted; JY, LL and LX performed surgical treatment; KX and XW provided gene sequencing analysis; SF reviewed and edited; SF and LX supervised the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding

Not applicable.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

Ethical approval was obtained from the institutional review board of the First Affiliated Hospital of USTC. Written informed consent was obtained from the patient.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Competing interests

The authors have no conflicts of interest to disclose.

Received: 16 September 2019 Accepted: 19 March 2020

Published online: 11 April 2020

References

1. Wang SK, Drucker NA, Gupta AK, Marshalleck FE, Dalsing MC. Diagnosis and management of the venous malformations of Klippel-Trenaunay syndrome. *J Vasc Surg Venous Lymphat Disord.* 2017;5(4):587–95.
2. Karadag A, Senoglu M, Sayhan S, Okromelidze L, Middlebrooks EH. Klippel-Trenaunay-weber syndrome with atypical presentation of cerebral cavernous Angioma: a case report and literature review. *World Neurosurg.* 2019;126:354–8.
3. Gupta Y, Jha RK, Karn NK, Sah SK, Mishra BN, Bhattarai MK. Management of femoral shaft fracture in Klippel-Trenaunay syndrome with external fixator. *Case Rep Orthop.* 2016;2016:8505038.
4. Tsaridis E, Papisoulis E, Manidakis N, Koutroumpas I, Lykoudis S, Banos A, Sarikloglou S. Management of a femoral diaphyseal fracture in a patient with Klippel-Trenaunay-weber syndrome: a case report. *Cases J.* 2009;2:8852.
5. Ranganathan K, Loder S, Agarwal S, Wong VW, Forsberg J, Davis TA, Wang S, James AW, Levi B. Heterotopic ossification: basic-science principles and clinical correlates. *J Bone Joint Surg Am.* 2015;97(13):1101–11.

6. Pacifici M. Acquired and congenital forms of heterotopic ossification: new pathogenic insights and therapeutic opportunities. *Curr Opin Pharmacol.* 2018;40:51–8.
7. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, McGuire AL, Nussbaum RL, O'Daniel JM, Ormond KE, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013;15(7):565–74.
8. Zhai J, Zhong ME, Shen J, Tan H, Li Z. Kyphoscoliosis with Klippel-Trenaunay syndrome: a case report and literature review. *BMC Musculoskelet Disord.* 2019;20(1):10.
9. Liu C, Song Y, Zhao J, Xu Q, Liu N, Zhao L, Lu S, Wang H. Elevated D-dimer and fibrinogen levels in serum of preoperative bone fracture patients. *Springerplus.* 2016;5:161.
10. Alwalid O, Makamure J, Cheng QG, Wu WJ, Yang C, Samran E, Han P, Liang HM. Radiological aspect of Klippel-Trenaunay syndrome: a case series with review of literature. *Curr Med Sci.* 2018;38(5):925–31.
11. Jacob AG, Driscoll DJ, Shaughnessy WJ, Stanson AW, Clay RP, Glociczki P. Klippel-Trénaunay syndrome: spectrum and management. *Mayo Clin Proc.* 1998;73(1):28–36.
12. Nauth A, Giles E, Potter BK, Nesti LJ, O'Brien FP, Bosse MJ, Anglen JO, Mehta S, Ahn J, Miclau T, et al. Heterotopic ossification in orthopaedic trauma. *J Orthop Trauma.* 2012;26(12):684–8.
13. Foley KL, Hebel N, Keenan MA, Pignolo RJ. Histopathology of periarticular non-hereditary heterotopic ossification. *Bone.* 2018;109:65–70.
14. Xu R, Hu J, Zhou X, Yang Y. Heterotopic ossification: mechanistic insights and clinical challenges. *Bone.* 2018;109:134–42.
15. Ramirez DM, Ramirez MR, Reginato AM, Medici D. Molecular and cellular mechanisms of heterotopic ossification. *Histol Histopathol.* 2014;29(10):1281–5.
16. Wang H, Lindborg C, Lounev V, Kim JH, McCarrick-Walmsley R, Xu M, Mangiavini L, Groppe JC, Shore EM, Schipani E, et al. Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling. *J Bone Miner Res.* 2016;31(9):1652–65.
17. Chen D, Liu S, Zhang W, Sun L. Rational design of YAP WW1 domain-binding peptides to target TGFbeta/BMP/Smad-YAP interaction in heterotopic ossification. *J Pept Sci.* 2015;21(11):826–32.
18. Lafont JE, Poujade FA, Padeloup M, Neyret P, Mallein-Gerin F. Hypoxia potentiates the BMP-2 driven COL2A1 stimulation in human articular chondrocytes via p38 MAPK. *Osteoarthr Cartil.* 2016;24(5):856–67.
19. Maeda Y, Tsuji K, Nifuji A, Noda M. Inhibitory helix-loop-helix transcription factors Id1/Id3 promote bone formation in vivo. *J Cell Biochem.* 2004;93(2):337–44.
20. Peterson JR, Eboda ON, Brownley RC, Cilwa KE, Pratt LE, De La Rosa S, Agarwal S, Buchman SR, Cederna PS, Morris MD, et al. Effects of aging on osteogenic response and heterotopic ossification following burn injury in mice. *Stem Cells Dev.* 2015;24(2):205–13.
21. Regard JB, Malhotra D, Gvozdenovic-Jeremic J, Josey M, Chen M, Weinstein LS, Lu J, Shore EM, Kaplan FS, Yang Y. Activation of hedgehog signaling by loss of GNAS causes heterotopic ossification. *Nat Med.* 2013;19(11):1505–12.
22. Zou YC, Yang XW, Yuan SG, Zhang P, Ye YL, Li YK. Downregulation of dickkopf-1 enhances the proliferation and osteogenic potential of fibroblasts isolated from ankylosing spondylitis patients via the Wnt/beta-catenin signaling pathway in vitro. *Connect Tissue Res.* 2016;57(3):200–11.
23. Chang EI, Chang EI, Thangarajah H, Hamou C, Gurtner GC. Hypoxia, hormones, and endothelial progenitor cells in hemangioma. *Lymphat Res Biol.* 2007;5(4):237–43.
24. Vahidnezhad H, Youssefian L, Uitto J. Klippel-Trenaunay syndrome belongs to the PIK3CA-related overgrowth spectrum (PROS). *Exp Dermatol.* 2016; 25(1):17–9.
25. Rached MT, Kode A, Xu L, Yoshikawa Y, Paik JH, Depinho RA, Kousteni S. FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. *Cell Metab.* 2010;11(2):147–60.
26. Zhang Y, Xiong Y, Zhou J, Xin N, Zhu Z, Wu Y. FoxO1 expression in osteoblasts modulates bone formation through resistance to oxidative stress in mice. *Biochem Biophys Res Commun.* 2018;503(3):1401–8.
27. Li L, Qi Q, Luo J, Huang S, Ling Z, Gao M, Zhou Z, Stiehler M, Zou X. FOXO1-suppressed miR-424 regulates the proliferation and osteogenic differentiation of MSCs by targeting FGF2 under oxidative stress. *Sci Rep.* 2017;7:42331.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://www.biomedcentral.com/submissions)

