COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Bockenheimer disease is associated with a *TEK* variant

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Abstract Bockenheimer disease is a venous malformation involving all tissues of an extremity. Patients have significant morbidity, and treatment is palliative. The purpose of this study was to identify the cause of Bockenheimer disease to develop pharmacotherapy for the condition. Paraffin-embedded tissue from nine individuals with Bockenheimer disease obtained during a clinically indicated operation underwent DNA extraction. Droplet digital polymerase chain reaction (ddPCR) was used to screen for variants most commonly associated with sporadic venous malformations (*TEK* [NM_000459.5:c.2740C > T; p.Leu914Phe], *PIK3CA* [NM_006218.4:c.1624G > A; p.Glu542Lys and NM_006218.4:c.3140A > G; p.His1047Arg]). ddPCR detected a *TEK* L914F variant in all nine patients (variant allele fraction 2%–13%). *PIK3CA* E542K and H1047R variants were not identified in the specimens. Sanger sequencing and restriction enzyme digestion confirmed variants identified by ddPCR. A pathogenic variant in the endothelial cell tyrosine kinase receptor *TEK* is associated with Bockenheimer disease. Pharmacotherapy targeting the TEK signaling pathway might benefit patients with the condition.

INTRODUCTION

Bockenheimer disease is an eponym describing a venous malformation (VM) involving most of the length of an extremity with all tissue planes affected (i.e., skin, subcutis, muscle, bone) (Kubiena et al. 2006). The condition is progressive and causes significant morbidity: pain, swelling, discoloration, phlebitis, ulceration, bleeding, joint stiffness, localized intravascular coagulopathy (LIC), pathologic fractures, and loss of function (Ali et al. 2020). Treatment includes compression, anticoagulants, sclerotherapy, and/or resection; amputation may be necessary. Bockenheimer disease is a sporadic condition, and its cause is unknown. The purpose of this study was to identify the etiology of Bockenheimer disease to develop improved treatments for patients. We hypothesized that because somatic activating variants in *TEK* and *PIK3CA* most commonly cause sporadic VMs, variants in these genes might also be responsible for Bockenheimer disease (Limaye et al. 2009, 2015).

RESULTS

Nine individuals with Bockenheimer disease of their arm or leg who underwent surgical excision were identified based on history, physical examination, and magnetic resonance (MR) imaging (Table 1; Fig. 1). The cohort included six females and three males. The disease

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Table 1. Bockenheimer disease cohort with a somatic *TEK* (NM_000459.5:c.2740C > T; p.Leu914Phe) variant

Patient	Age at resection	Sex	Location	VAF (%)
1	3	Female	Left leg	7.8
2	9	Female	Left arm	8.8
3	10	Male	Left arm	10.1
4	13	Female	Right arm	12.8
5	18	Male	Right arm	13.2
6	20	Female	Left leg	2.0
7	21	Male	Right arm	10.3
8	38	Female	Left arm	12.2
9	46	Female	Right arm	6.2

(VAF) Variant allele frequency.

affected the right arm ($n = 4$), left arm ($n = 3$), or left leg ($n = 2$). Droplet digital polymerase chain reaction (ddPCR) identified a *TEK* L914F variant (NM_000459.5:c.2740C > T) in all nine individuals (Table 2). The variant allele frequency (VAF) ranged from 2% to 13%. None of the tissue specimens contained either of the two *PIK3CA* variants that were tested. Patients 4, 5, and 8 with the highest VAFs had the *TEK* L914F variant confirmed with HpyAV digestion. The *TEK* L914F variant also was confirmed in Patient 5 by Sanger sequencing.

DISCUSSION

In 1907 Phillip Bockenheimer reported an adult male who had progressive upper extremity enlargement with dilated veins, ulceration, pain, and loss of function (Kubiena et al. 2006). Bockenheimer noted ectasia of all veins and that the bones were involved; he named the condition "genuine (congenital) diffuse phlebectasia" (Kubiena et al. 2006). Since the biological classification of vascular anomalies was established in 1982 (Mulliken and Glowacki 1982), the patient described by Bockenheimer was recognized to have a VM. The

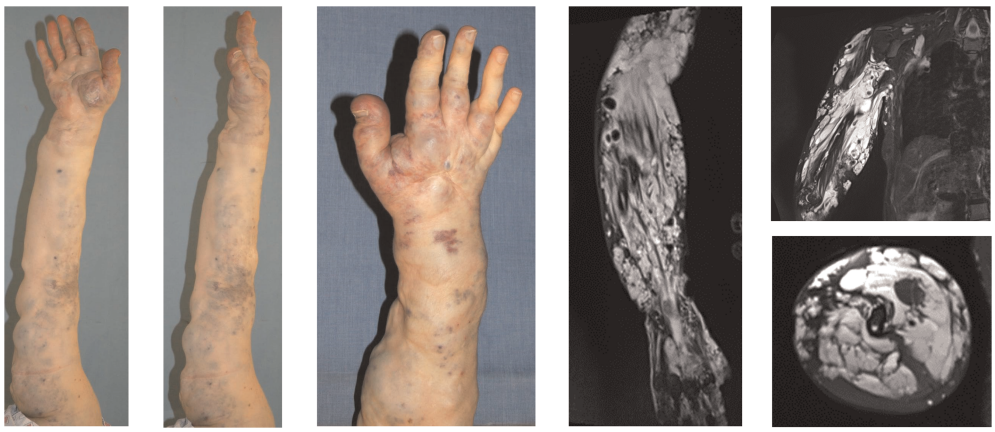

Figure 1. Adult female with Bockenheimer disease of the right arm (Patient 9). The patient has a diffuse venous malformation affecting the entire limb; magnetic resonance images show that all tissue planes are affected. Resected affected tissue contained a somatic *TEK* (NM_000459.5:c.2740C > T; p.Leu914Phe) variant (variant allele frequency [VAF] 6.2%).

Table 2. Variant table

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
TEK	Chr 9: 27212760 (GRCh38)	NM_000459.5:c.2740C > T	p.Leu914Phe	Somatic heterozygous	N/A	Pathogenic

Bockenheimer eponym has been used to describe a VM that (1) affects most of the length of an extremity and (2) extends from the skin to the bone (Kubiena et al. 2006; Ali et al. 2020). Bockenheimer disease is recognized by the National Institutes of Health in the ClinVar database and by the European Union in the Ontology Lookup Service (MONDO_ 0016311).

VM *TEK* variants are associated with distinct phenotypes. We believe it is important to continue to use the Bockenheimer eponym because it describes a unique condition that facilitates communication and patient management. A germline *TEK* variant (NM_000459.4: c.2545C > T; p.Arg849Trp) causes cutaneomucosal VMs (Vikkula et al. 1996). Blue rubber bleb nevus syndrome (multifocal VMs of the skin, soft tissue, and gastrointestinal tract) results from somatic *TEK* variants occurring in *cis* (NM_000459.5:c.3314C > A; 3316A > C; p.Thr1105Asn;Thr1106Pro and NM_000459.4:c.2690A > T; 2744G > T; p.Tyr897Phe; Arg915Leu) (Soblet et al. 2017). The most common VMs are somatic, localized, and unifocal: These VMs with a *TEK* (L914F) variant typically affect the skin, whereas lesions containing a *PIK3CA* variant usually are subcutaneous (Limaye et al. 2009, 2015). The extent of the VM does not correlate with its VAF (Limaye et al. 2009), similar to our finding that Bockenheimer disease can result from a wide range of VAFs.

TEK is an endothelial cell tyrosine kinase receptor for vascular growth factors responsible for angiogenesis and vasculogenesis (Limaye et al. 2009). *TEK* variants affect the cytoplasmic kinase domain of the receptor. R849W blocks phosphatase activity preventing the kinase from being turned off, whereas L914F constitutively increases phosphorylation in the absence of ligand activation (Limaye et al. 2009; Du et al. 2017). *TEK* variants cause endothelial cells to grow in an abnormal pattern. Instead of a cobblestone monolayer, cells are elongated, overlap, and have decreased extracellular fibronectin (Nätynki et al. 2015). It is possible that the *TEK* L914F variant occurred earlier during embryogenesis or in a multipotent cell type to cause Bockenheimer disease compared to localized VMs. The *TEK* variant also may predispose cells to develop a second hit causing the Bockenheimer phenotype (Dekeuleneer et al. 2020). This possibility could be determined by searching the tissue for different TEK variants as well as variants in other genes involved in vascular signaling.

Patients with Bockenheimer disease typically have significant morbidity because the VM is extensive. Sclerotherapy and resection usually are only able to target the most problematic areas, such as localized sites of pain. Unfortunately, recurrence and progression of symptoms are common. Off-label use of sirolimus, an inhibitor of mTOR in the PIK3CA signaling pathway, has demonstrated efficacy in animal models and patients with VMs (Boscolo et al. 2015; Salloum et al. 2016; Hammer et al. 2018). Because we now know that Bockenheimer disease is associated with a pathologic *TEK* variant, patients may be candidates for sirolimus or other targeted therapies affecting TEK signaling.

METHODS

Our Vascular Anomalies Center database was searched for patients diagnosed with Bockenheimer disease who underwent a clinically indicated operative procedure of the limb. Formalin-fixed paraffin-embedded (FFPE) scrolls were obtained from the Department

of Pathology. DNA was extracted using the Agencourt FormaPure DNA kit (Beckman Coulter). Patient-matched control tissue was unavailable. Variants in genes most frequently associated with sporadic VMs were screened using ddPCR: (1) *TEK* (NM_000459.5: c.2740C > T; p.Leu914Phe), (2) *PIK3CA* (NM_006218.4:c.1624G > A; p.Glu542Lys), and (3) *PIK3CA* (NM_006218.4:c.3140A > G; p.His1047Arg) (Limaye et al. 2009, 2015). A VAF > 1.0% was considered a true positive. Variants identified by ddPCR were confirmed with Sanger sequencing in one patient. Because the *TEK* variant disrupts a restriction site for the enzyme HpyAV (New England BioLabs), PCR fragments for three patients were digested with HpyAV as another method to confirm the presence of the *TEK* variant: (forward primer [FP]: 5′-GCCCCATGGAAATCTTCTGGAC-3′; reverse primer [RP]: 5′-CAGCGAAGTGA AGGAGCTGCTG-3′. The FP was designed to mutate a second HpyAV site, 13 bp upstream from the TEK variant (CCTTC to TCTTC). Digestion of the resulting 123-bp PCR fragment with HpyAV results in 35- and 88-bp fragments for a wild-type PCR fragment, whereas a PCR fragment containing the TEK variant will not be cut.

PCR primers and fluorescent probes for detecting wild-type (WT) and mutant *TEK* L914F alleles were designed: (1) FP 5′-TACGCGCCCCATGGAAACCT-3′, (2) RP 5′-ACAGT GTGGACGGGGTGCTA-3′, (3) WT probe 5′-5HEX/ACTTCCTTCGCAAGAGCCGTGT/-3′, (4) mutant probe 5′-/56-FAM/TTCTTTCGCAAGAGCCGTGTG/-3′. *PIK3CA* E542K primers and probes included (1) FP 5′-CAGCTCAAAGCAATTTCTAC-3′, (2) RP 5′-CACTTACC TGTGACTCCAT-3′, (3) WT probe 5′-/5HEX/CTGAAATCA/ZEN/CTGAGCAGGAGA/-3′, (4) mutant probe 5′-/56-FAM/CTAAAATCA/ZEN/CTGAGCAGGAG/-3′. *PIK3CA* H1047R primers and probes were (1) FP 5′-AACTGAGCAAGAGGCTTTGG-3′, (2) RP 5′-TGTGTG GAAGATCCAATCCA-3′, (3) WT probe 5′-5HEX/TGCACATCA/ZEN/TGGTGGCTGGA/-3′, (4) mutant probe5′-/56-FAM/TGATGCACG/ZEN/TCATGGTGGCT/-3′. For each ddPCR reaction we used 45 ng of template DNA. ddPCR was performed with a QX200 Droplet Generator, QX200 Droplet Reader, and QuantaSoft Software (Bio-Rad).

ADDITIONAL INFORMATION

Data Deposition and Access

The generated data set was deposited to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV001739510.

Ethics Statement

The Committee on Clinical Investigation at Boston Children's Hospital approved this study. All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Author Contributions

C.L.S., D.J.K., and P.J.S. curated the data, administered and visualized the project, provided software, reviewed and edited the manuscript, and performed formal analysis, methodology, validation, and investigation. W.E. curated the data, administered and visualized the project, provided software, reviewed and edited the manuscript, and performed

conceptualization, formal analysis, methodology, validation, and investigation. A.A.-I. curated the data, provided software, visualized the project, reviewed and edited the manuscript, and performed formal analysis, methodology, and validation. J.U. curated the data, visualized, supervised, and validated the project, reviewed and edited the manuscript, and provided resources. A.K.G. curated the data, administered and visualized the project, provided software, wrote the original draft and reviewed and edited the manuscript, provided resources, acquired funding, and performed conceptualization, formal analysis, methodology, supervision, validation, and investigation.

Competing Interest Statement

The authors have declared no competing interest.

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