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CASE REPORT

USP6 Gene Rearrangement by FISH Analysis in Cranial Fasciitis: A Report of Three Cases

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Abstract

Cranial fasciitis (CF) is an uncommon benign myofibroblastic proliferation involving the soft and hard tissues of the cranium. It typically occurs in the pediatric population with a male predilection (male-to-female ratio 1.5:1). The clinical presentation is usually a rapidly expanding, painless nodule. Bone erosion may be appreciated radiographically. Histopathologic sections of CF show plump, fibroblast-like cells with pale, oval shaped nuclei and prominent nucleoli in a fibrous or myxoid background. Growth is self-limited and surgical excision is considered curative. Due to these features, CF is thought to be a variant of nodular fasciitis (NF). As with NF, CF may mimic a sarcomatous process and pose a diagnostic challenge to clinicians and pathologists alike. Erickson-Johnson et al. identified rearrangements of the ubiquitin-specific protease 6 (*USP6*) gene in 44 of 48 cases of NF. *MYH9* was the fusion partner in 12 of these cases. To date, the molecular profile of CF has not been studied. Here we present the molecular findings in three cases of CF identified at our institution. Each case was subjected to fluorescence in-situ hybridization with appropriate negative controls. Two of three cases were positive for the *USP6* gene rearrangement. The third case failed to hybridize, likely related to nucleic acid damage secondary to decalcification. Negative controls did not demonstrate the genetic rearrangement. These findings warrant further investigation of the *USP6* gene rearrangement in CF, as it may prove helpful as a diagnostic adjunct in challenging cases.

Keywords Cranial fasciitis · Nodular fasciitis · Gene fusion · FISH · USP6 · MYH9

Introduction

Cranial fasciitis (CF) is a benign myofibroblastic proliferative condition of the soft and hard tissues of the cranium. It is considered a rare variant of nodular fasciitis (NF), which typically presents as a rapidly-growing subcutaneous mass in the upper extremities, trunk, and head and neck of children and young adults [1]. CF usually presents in children under the age of two and erodes the outer table of the skull. Since it was first described in 1980 by Lauer et al., 72 cases have been reported in the English literature [2]. While CF is observed in patients across all age spectrums, it most often presents in the pediatric population. It develops more commonly in males when compared to females, with a male-to-female ratio of 1.5:1. Patients classically present with a two-to-four-week history of a firm, non-painful, and rapidly enlarging scalp lesion, usually localized to the subcutaneous tissue or galea aponeurotica [3, 4]. While CF may develop anywhere on the cranium, patients frequently present with lesions in the temporal and parietal regions [5]. Transcranial invasion is a potential complication [6, 7]. The exact

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etiology of CF remains poorly understood, although a history of trauma is not uncommon.

A computerized tomography (CT) scan of the head is useful in identifying underlying skeletal muscle involvement [8]. In more aggressive cases, lytic bone lesions are identified where the tumor erodes into the cranium [9]. Brain magnetic resonance imaging (MRI) allows for better characterization of CF when compared to CT. T1-weighted MRI classically demonstrates hypointensity and vivid enhancement with a non-enhancing central region within the scalp mass. T2-weighted imaging reveals hyperintensity within the central non-enhancing area [10].

Diagnosis of CF is based on histopathologic examination of biopsied tissue specimens. The histologic features include proliferating fibroblasts and myofibroblasts arranged in short, loose fascicles with a surrounding a fibromyxoid stroma (Fig. 1). The cells have a bland spindle or stellate appearance and are often organized in a storiform or nodular growth pattern [11]. Multinucleated giant cells, abundant vascular proliferation, and a mixed inflammatory cell infiltrate may be present [12]. A high degree of cellularity and brisk mitotic activity often impart a sarcomatous appearance, making the distinction of CF from other aggressive spindle-cell lesions a potential challenge [13].

Three histopathological variants of CF have been described: myxoid, cellular and fibrous [14]. If metaplastic bone formation is present, a diagnosis of myositis ossificans may be rendered. This condition has CF-like features and is also positive for a gene rearrangement of ubiquitin-specific protease 6 (*USP6*). Myositis ossificans, NF, and CF are related tumors on a spectrum [15].

The immunohistochemical profile of CF is non-specific, with proliferative fibroblasts showing non-specific positivity for vimentin and smooth muscle actin (SMA). More recently, select cases of CF have proved to be negative for beta-catenin, with staining limited to cytoplasmic positivity [16].

The standard treatment for CF is complete surgical resection [3]. An incisional biopsy is often performed for diagnostic purposes. An excisional biopsy may be performed as both a diagnostic and therapeutic modality in certain clinical scenarios. Such instances include a high index of suspicion for a benign diagnosis, limited size of the lesion, and capability for direct closure of the wound [11]. Local recurrence is uncommon following surgical excision. Lee et al. described the use of an intralesional triamcinolone acetonide injection as an alternative treatment modality in a single case. The patient received intralesional triamcinolone acetonide injection twice daily for 10 days. There was no evidence of disease persistence or recurrence 4 months post-treatment [17].

Recently, molecular studies have demonstrated a consistent rearrangement of the *USP6* gene in NF.

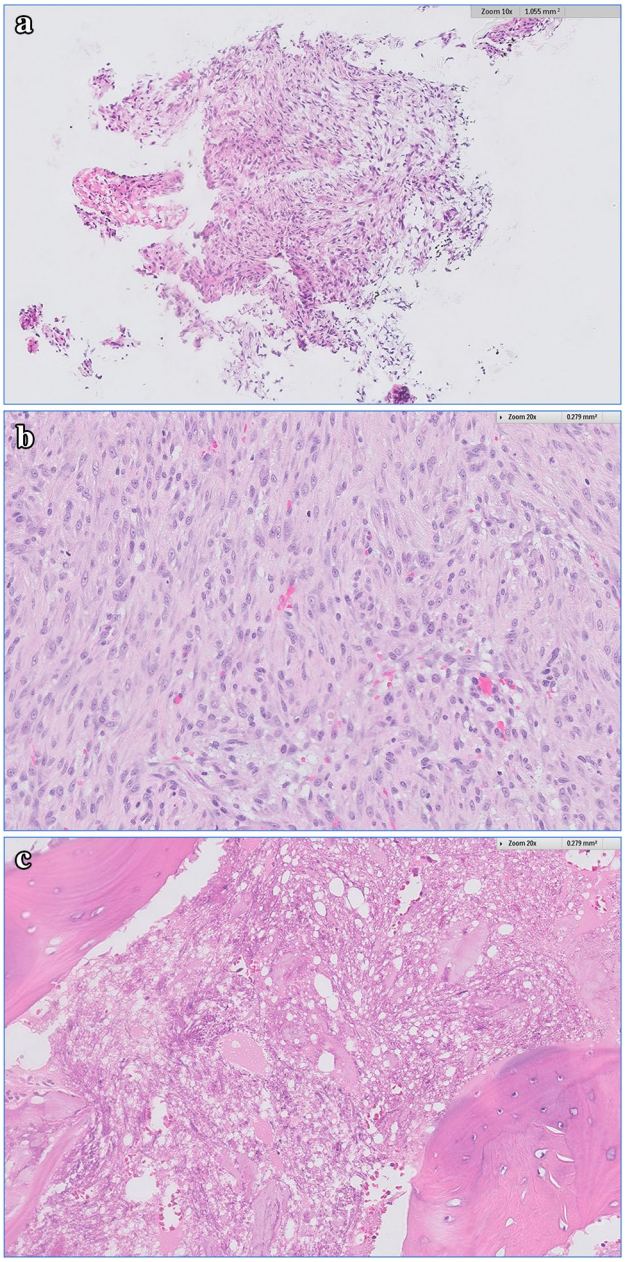

Fig. 1 **a** H&E, ×10 magnification. Proliferating spindle-shaped fibroblasts and myofibroblasts arranged in loose fascicular and vague storiform patterns in a fibromyxoid stroma. **b** H&E stain, ×200 original magnification. High magnification demonstrating a dense spindle cell population with cells possessing large nuclei and conspicuous nucleoli. **c**. H&E, ×20 magnification. CF showing a fibrous stroma with haphazardly arranged spindled cells with surrounding bone

Erickson-Johnson et al. studied 48 cases of NF and found 44 to be positive for the *USP6* rearrangement. *MYH9* was identified as the fusion partner in 12 of the 44 cases [1]. The *USP6* gene rearrangement was not identified in control cases, which included benign and malignant neoplasms of both mesenchymal and epithelial in origin [1]. More recently, the same authors published a review of

USP6-induced neoplasms [18]. They reported a sensitivity and specificity of 93% and 100%, respectively, for *USP6* rearrangements as a diagnostic marker for NF as confirmed by fluorescence in-situ hybridization (FISH) [18].

Given the similarities in biologic behavior and histopathology between NF and CF, we suspected they might also share *USP6* rearrangements. Our aim in this study was to evaluate archived cases of CF for this molecular finding via FISH analysis.

Materials and Methods

Exempt status was obtained from the North Shore-Long Island Jewish Hospital Health System Institutional Review Board (IRB#: 15-060). Three cases of CF were identified and retrieved from the surgical pathology archives. All cases showed classic histopathological features composed of a subcutaneous cellular spindle cell proliferation exhibiting prominent mitotic activity, occasional microcystic changes, extravasated erythrocytes, and an inflammatory infiltrate [19]. Negative controls included a mandibular myofibroma and a pharyngeal rhabdomyosarcoma.

FISH for the *USP6* rearrangement was performed at the Mayo Clinic Sequencing Core using 5-micrometer thick sections of formalin fixed, paraffin embedded tissue samples from the five specimens listed above. Identification of the Mayo Clinic Home-brew 5′ *USP6* DNA (clones RP11-198F11, RP11-115H24, and RP11-124C16) labeled with SpectrumOrange dUTP (Abbott Molecular/Vysis Products) and home-brew 3′ *USP6* DNA (clones CTD-2367F23, RP11-457I18, RP11-1140D18, and RP11-373N8) labeled in SpectrumGreen dUTP (Abbott Molecular/Vysis Products) were combined as one probe set (Fig. 2). The break-apart (BAP) probe set was applied to individual slides, hybridized, and washed according to the PAT reduced pepsin FISH protocol. At least 50 non-overlapping specimen nuclei were analyzed for the *USP6* probe by two technicians.

Results

Clinical and molecular characteristics of CF ($n=3$) are summarized in Table 1. Briefly, each patient with CF was male and ranged in age from 1 to 7 years (mean 3-years). Each case presented as a scalp lesion, with one case demonstrating bone erosion (see Table 1). Histologically, the tumors showed myofibroblasts arranged in short, loose fascicles in a fibromyxoid stroma with classic myxoid degeneration, extravasation of erythrocytes and lymphocytes, and often

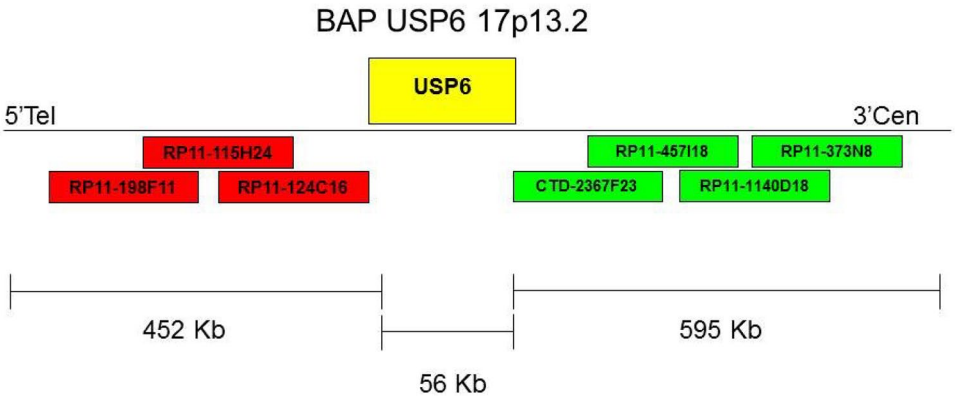

Fig. 2 Depiction of the home-brew 5′ *USP6* DNA labeled with SpectrumOrange dUTP (Abbott Molecular/Vysis Products) and home-brew 3′ *USP6* DNA labeled in SpectrumGreen dUTP (Abbott Molecular/Vysis Products) were combined as one probe set. The break-apart (BAP) probe set was applied to individual slides, hybridized, and washed according to the PAT reduced pepsin FISH protocol

Table 1 Clinical and molecular features of three CF cases

Case	Sex	Age (years)	Clinical presentation	Procedure	Diagnosis	FISH results
1	M	2	Posterior scalp mass	Excisional biopsy	Spindle cell proliferation consistent with cranial fasciitis	Positive 46 break-apart signals out of 100 nuclei counted
2	M	7	Right parietal scalp mass	Excisional biopsy	Consistent with cranial fasciitis	Positive 51 break-apart signals out of 100 nuclei counted
3	M	1	Frontotemporal suture bony erosion & soft tissue mass	Excisional biopsy	Cranial fasciitis	Hybridization failure

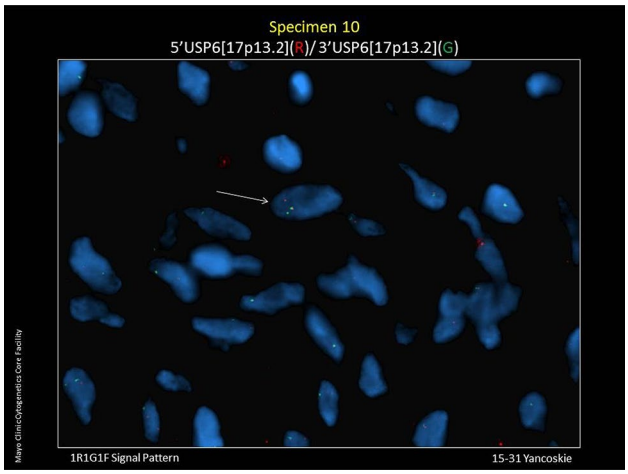

Fig. 3 Specimen Case 1; 5′ *USP6* DNA labeled with SpectrumOrange dUTP and 3′ *USP6* labeled in SpectrumGreen dUTP. Break-apart signal seen in three tumor nuclei indicating *USP6* gene rearrangement

stromal keloidal collagen. The hemorrhage pattern appeared intralesional rather than perivascular. Two cases were positive for the rearrangement of *USP6* gene and one case failed to hybridize (Fig. 3). The myofibroma was negative for the rearrangement of *USP6* gene. While the rhabdomyosarcoma was negative for the rearrangement, it did show amplification in the *USP6* region.

Discussion

CF is a rare tumor of the soft and hard cranial tissues that occurs more commonly in males than females, typically in the pediatric population, and has a predilection for the temporal and parietal regions. It is characterized by a rapidly enlarging, non-painful mass. The histopathologic findings show proliferating bland spindle or stellate fibroblasts and myofibroblasts arranged in short, loose fascicles in a fibromyxoid stroma. In this report, we present three cases of classic CF. FISH analysis revealed rearrangement of the *USP6* gene in 2 of the 3 cases.

Erickson-Johnson et al. first reported *USP6* gene rearrangement on chromosome 17p13 as a recurrent and specific finding in NF [1]. This gene codes for a deubiquitinizing enzyme involved in several cellular processes, including intracellular trafficking, inflammatory signal response, and protein metabolism [1]. This gene rearrangement was not observed in normal control tissue. Moreover, the authors subsequently analyzed a wide variety of benign and malignant mesenchymal and epithelial neoplasms which also lacked this genetic alteration. The authors estimate a 93% sensitivity and 100% specificity of *USP6* by FISH as a diagnostic marker for NF [1]. Given the regressive nature of NF, this genetic hallmark is the first example of a self-limited condition characterized by a recurrent fusion event. This finding challenges the conventional ideology that non-random genetic translocations are exclusively associated with sustained neoplastic processes [20, 21].

The most common somatic fusion partner of *USP6* is *MYH9* on chromosome 22q13.1. This gene encodes structural cytoskeletal myosin that is highly expressed in fibroblasts, endothelial cells, leukocytes, and monocytes [20]. Identifying the t(17;22)(p13;q13) translocation of *MYH9-USP6* formation via traditional cytogenetic analysis is challenging, as the chromosomal breakpoints are located at the ends of these chromosomes. A recent study series conducted by Amary et al. detected *USP6* rearrangement with the *MYH9* gene fusion partner in 31 of 34 NF cases by FISH, translating to a sensitivity of approximately 91% [21]. Similar findings were reported by Patel and colleagues, who observed the *USP6* gene rearrangement in 20 of 26 cases of NF. Of the 20, 14 were also tested for *MYH9-USP6* translocation via reverse transcription polymerase chain reaction (RT-PCR), and 8 of the 14 were found to be positive [22].

Detection of the *USP6* gene rearrangement can offer diagnostic value when encountering tumors possessing overlapping histopathology, such as desmoid tumors, myofibromas or other sarcomas. These entities may show a varying amount of fibroblasts in a myxoid stroma and storiform architectural growth pattern, making the distinction between self-limited conditions and malignant tumors challenging. We have presented three cases of CF that exhibited an aggressive clinical course, two of which were found to harbor *USP6* gene mutation by FISH analysis. Although these findings demonstrate and confirm the characteristic genetic alteration in CF, further studies will be essential in establishing other possible genetic alterations.

In conclusion, CF is a rare condition that should be considered in the differential diagnosis of young children or infants presenting with rapidly enlarging scalp or skull masses. Our results demonstrate that the *USP6* gene rearrangement is present in select cases of CF. This finding warrants further investigation to determine whether the *USP6* gene rearrangement may be a useful diagnostic adjunct.

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