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BRIEF REPORT

Localized Hypertrophic Neuropathy as a Neoplastic Manifestation of *KRAS*-Mediated RASopathy

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Abstract

Localized hypertrophic neuropathy is a rare Schwann cell proliferation that usually affects single nerves from the extremities, and it is of unclear etiology in its pure form. RASopathies are a defined group of genetic diseases with overlapping clinical features, usually secondary to germline mutations in genes encoding either components or regulators of the RAS/MAPK pathway. Herein, we report an 11-year-old boy presenting with café au lait spots and right leg length discrepancy. A fascicular nerve biopsy of the tibial nerve demonstrated a Schwann cell proliferation with prominent onion-bulb formation, satisfying criteria for localized hypertrophic neuropathy. Molecular genetic analysis demonstrated identical *KRAS* mutations (c38_40dupGCG) in the peripheral nerve lesion and melanocytes from café au lait spots, but not in blood, supporting a diagnosis of a KRAS-mediated rasopathy with mosaicism. Immunohistochemical staining in the peripheral nerve lesion demonstrated strong pERK staining consistent with downstream MAPK pathway activation. This report suggests that at least a subset of localized hypertrophic neuropathies are bonafide, well-differentiated Schwann cell neoplasms developing through oncogenic RAS signaling, which provides new insights into the controversial entity historically known as localized hypertrophic neuropathy.

Key Words: KRAS, Localized hypertrophic neuropathy, Peripheral nerve, RASopathy, Schwann cell.

INTRODUCTION

Localized hypertrophic neuropathy (LHN) is a rare Schwann cell proliferation that usually affects single nerves from the extremities. Histologically, it is characterized by "onion bulb" formation, composed of layers of Schwann cell processes and historically interpreted as a reactive entity (1, 2). However, it was understood over time that a subset, if not most of these cases, represented a cellular proliferation with a perineurial phenotype (i.e. intraneural perineurioma) (3, 4). The latter is now recognized as a true neoplasm with clonal mutations in *TRAF7* or *NF2* (5). Therefore, true LHN can be currently defined as a mass-like Schwann cell lesion in the absence of a generalized neuropathy, and remains very rare after excluding cases that represent intraneural perineuriomas. Morphologic entities in the differential diagnosis include inherited neuropathies associated with onion bulb formation, such as Charcot-Marie-Tooth. Schwannosis are rare localized nonneoplastic Schwann cell proliferations in the spinal cord, which may affect older patients or develop in the context of neurofibromatosis type 2. However, clinical presentation, morphology, immunophenotype, and distribution of these lesions are different from LHN.

RASopathies are a defined group of genetic diseases with overlapping clinical features, usually secondary to germline mutations in genes encoding either components or regulators of the RAS/MAPK pathway. Abnormalities of this pathway comprise one of the largest known groups of developmental syndromes, affecting ~1 in 1000 individuals (6). The best-known syndromes related to this pathway and their main alterations include neurofibromatosis type 1 (NF1), capillary malformation-arteriovenous malformation, Noonan and Noonan with multiple lentigines, Costello, Legius, and cardiofacio-cutaneous syndromes. However, the list of genetic alterations and phenotypes under this common biologic umbrella continues to grow. Herein, we present a case of extensive LHN developing in a patient satisfying clinical and genetic criteria of mosaic RASopathy.

MATERIALS AND METHODS

Pathologic Analysis

Clinical information was abstracted from electronic chart records. All available imaging, including magnetic resonance (MR) imaging, was reviewed. All available histologic

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slides were reviewed. Immunohistochemistry was performed using antibodies targeting EMA (Cellmarque, clone E29), GLUT1 (Cellmarque, polyclonal), S100 (Ventana, clone 4C4.9), neurofilament protein (SM31, Sternberger), phospho-ERK (Cell Signaling, D13.14.4E, XP Rabbit mAb; 1:250), and Ki-67 (Ventana, clone 30-9). One piece of peripheral nerve tissue was glutaraldehyde-fixed and epon-embedded. Semithin sections were examined and blocks with onion bulbs subjected to electron microscopy analysis.

Molecular Analysis

Peripheral nerve sheath tumor targeted next generation sequencing (NGS) and copy number analysis protocol was performed at the University of Alabama, Birmingham for the following genes: *KRAS* (exon 2), *PTPN11*, *NF1*, *NF2*, *SMARCB1*, and *LZTR1*. DNA was extracted from fresh lesional tissue from peripheral nerve, as well as cultured Schwann cells from the peripheral nerve lesion and melanocytes from one pigmented skin spot. The average coverage for NGS testing is ∼1600× with >98% of the coding region ≥350× and >99% ≥200×. This allows for the detection of low-level mosaicism, down to 3%–5% variant allele fraction with 95% confidence. DNA extracted from blood lymphocytes was tested by Sanger sequencing. Additional details on the testing methods may be gathered from the UAB laboratory website (https://www.uab.edu/medicine/genetics/medical-genomics-laboratory/testing-services/nf1-legius-syndrome-and-rasopathies/ras-ng).

RESULTS

Clinical History

The patient was an 11-year-old boy with no significant personal or family medical history. He presented with right leg length discrepancy for one year, as well as 4 subtle café au lait spots observed under Wood's lamp involving the face, trunk and extremities bilaterally. Radiographs showed bilateral, multiple stress fractures of the lower limbs. MR imaging showed diffuse, smooth enlargement of the lumbosacral plexus and right sciatic nerve extending to the tibial, common peroneal, and deep and superficial peroneal nerves throughout their visualized course without associated enhancement or bulbous enlargement (Fig. 1). The patient underwent corrective surgery and a concurrent fascicular biopsy of the tibial nerve was performed.

Pathology

The 0.6-cm specimen consisted of a nerve fascicle showing sclerosis of the epineurium and slight irregularity of the perineurium. There were concentric whorls in the form of "onion bulbs" (Fig. 2). Longitudinal sections demonstrated areas of increased cellularity, but mitotic activity was inconspicuous. Immunohistochemical stain for S100 was strongly positive and outlined the onion bulbs (Fig. 2). EMA was negative and neurofilament protein highlighted sparse, residual axons, some in the center of the onion bulbs (Fig. 2), suggesting that the Schwann cell process did not involve every axon. GLUT1 showed weak, nonspecific immunoreactivity, with strong labeling limited to erythrocytes which served as internal positive control. Strong immunoreactivity for phospho-ERK was detected (Fig. 2). This antibody recognizes levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated at 2 relevant sites (Thr202 and Tyr204) of Erk1 and (Thr185 and Tyr187) of Erk2, as well as singly phosphorylated at Thr202. This provides evidence of MAPK pathway activation, downstream of RAS. We have previously detected similar immunoreactivity in neoplasms associated with other RASopathies (Noonan) (7) or activating somatic *BRAF* alterations (8). Ki67 labeling index was very low (Fig. 2). Electron microscopy examination was limited by a predominant longitudinal orientation of the sections examined. However, numerous Schwann cell processes were concentrically arranged in areas, and axonal loss was also detected (Fig. 2), which correlated with nerve conduction loss identified intraoperatively. This suggests a degree of chronicity with associated partial axonal loss in the lesion and provided further support for the Schwann cell nature of the process.

Molecular Genetics

NGS molecular analysis performed in fresh peripheral nerve lesional tissue identified a *KRAS* duplication (c38_40dupGCG, mutant allele fraction of ∼16%, 192 mutation reads/∼1150 wild type reads). The same alteration was found in cultured Schwann cells from the nerve lesion and at a low level in cultured melanocytes from the café au lait spot. No genetic alterations were detected in blood by Sanger sequencing (1040 reads over ∼5 amplicons). The same *KRAS* alteration has been reported in other neoplasms (COSV55499554), including tumors of the large and small bowel (9). The potential functional impact of the in-frame insertion variant was ascertained by Ensembl's Variant Effect Predictor (VEP) (10) and Sorting Intolerant From Tolerant (SIFT) programs (11). VEP predicted a moderate effect whereas SIFT predicted a damaging effect on gene function (Supplementary Data Table S1). As described above, strong immunohistochemical staining for phosphor-ERK, a known downstream mediator of the MAPK pathway, was detected in lesional tissue consistent with an activating mutation. Taken together, these findings supported a diagnosis of LHN developing in the context of a KRAS-driven RASopathy with possible mosaicism.

DISCUSSION

LHN is a rare, unique lesion of the peripheral nerve that has undergone nosologic change through the past several decades. The histological and imaging based differential diagnosis of LHN includes primarily intraneural perineurioma, but also neurofibroma with onion bulb-like formation, and other hereditary neuropathies like Charcot-Marie-Tooth. Additionally, most of the lesions that were diagnosed in the past as LHN are now believed to represent intraneural perineuriomas (3, 4). In contrast to true LHN using a strict definition of a Schwann cell lesion, perineuriomas are EMA-positive and S100-negative neoplasms. Even though neurofibromas with

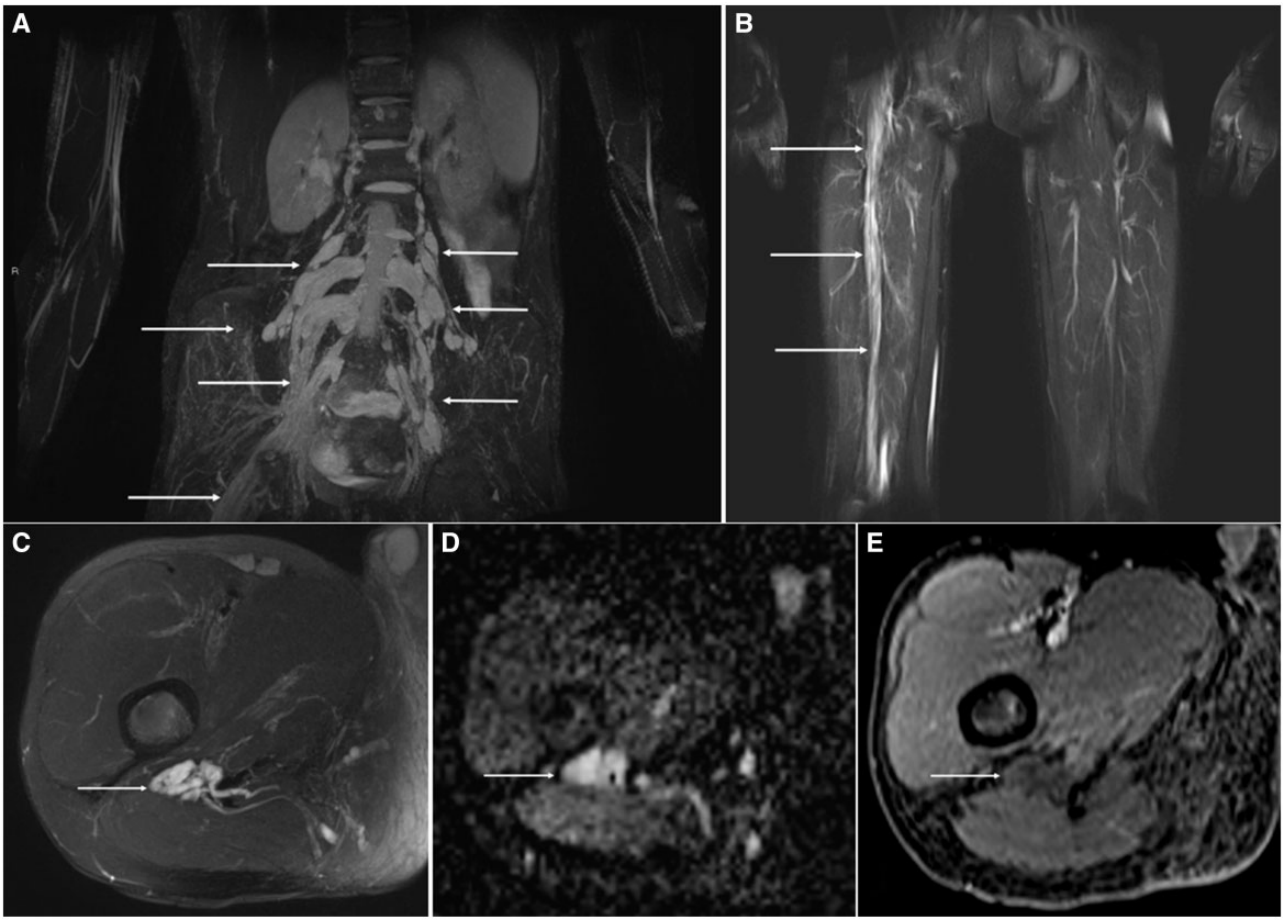

FIGURE 1. Imaging features of localized hypertrophic neuropathy in RASopathy. Coronal Short tau inversion recovery (STIR) Sampling Perfection with Application optimized Contrasts using different flip angle Evolution (SPACE) maximum intensity projection (MIPs) through the pelvis **(A)** and thighs **(B)** shows multifocal, bilateral lumbar nerve root thickening (arrows) extending into the right lumbosacral plexus and sciatic nerve root (arrow). Axial T2 fat suppressed (FS) image through the proximal thigh **(C)** shows marked thickening of the sciatic nerve (arrow) without a discrete mass. Note, the absence of imaging features of a peripheral nerve tumor such as a target sign. Apparent diffusion coefficient map through the right thigh **(D)** also reveals marked thickening of the sciatic nerve (arrow) without a discrete mass or restricted diffusion to suggest a hypercellular neoplasm. Axial T1-FS post contrast image through right thigh **(E)** also reveals marked thickening of the sciatic nerve (arrow) without internal enhancement.

onion bulb-like formation are also positive for S100, they display their classic diffuse or plexiform morphology, with onion bulb-like formation present only focally. Schwannosis, although having some histologic similarities, is typically limited to the spinal cord in older patients or those with NF2, and therefore the distribution is different from LHN.

Molecular analysis can be also helpful in characterizing these lesions. No alterations were identified in the *NF1* gene in the current case, but rather a pathogenic mutation in *KRAS* was found. Neurofibromas uniformly carry inactivating mutations in *NF1* (12), and therefore, we interpreted this Schwann cell proliferation as most compatible with LHN. Molecular analyses of this lesion are lacking in the literature given its rarity, which is now accentuated by the exclusion of intraneural perineurioma, that was likely responsible for most cases previously described as LHN in the literature.

Of interest, the patient had a *KRAS* alteration not only in the peripheral nerve lesion, but also in samples from cutaneous melanocytes while being negative in blood, which supported a mosaic presentation of a RASopathy, a heterogeneous and expanding group of genetic disorders with variable genetic drivers that have in common alterations in the MAPK/RAS pathway (6, 13). The patient's activating *KRAS* gene mutation could lead to hyperactivation of RAS signaling cascades resulting in an increased risk for the development of other tumors in the future, and may thus require regular clinical and radiographic surveillance, as is done routinely for other syndromes in this category. The best characterized ones are NF1, caused by *NF1* mutations, and Noonan syndrome, caused by several mutations, including *PTPN11*. More recently, Legius syndrome was recognized as a member of this group, a disorder resulting from mutations in the Sprouty protein family and

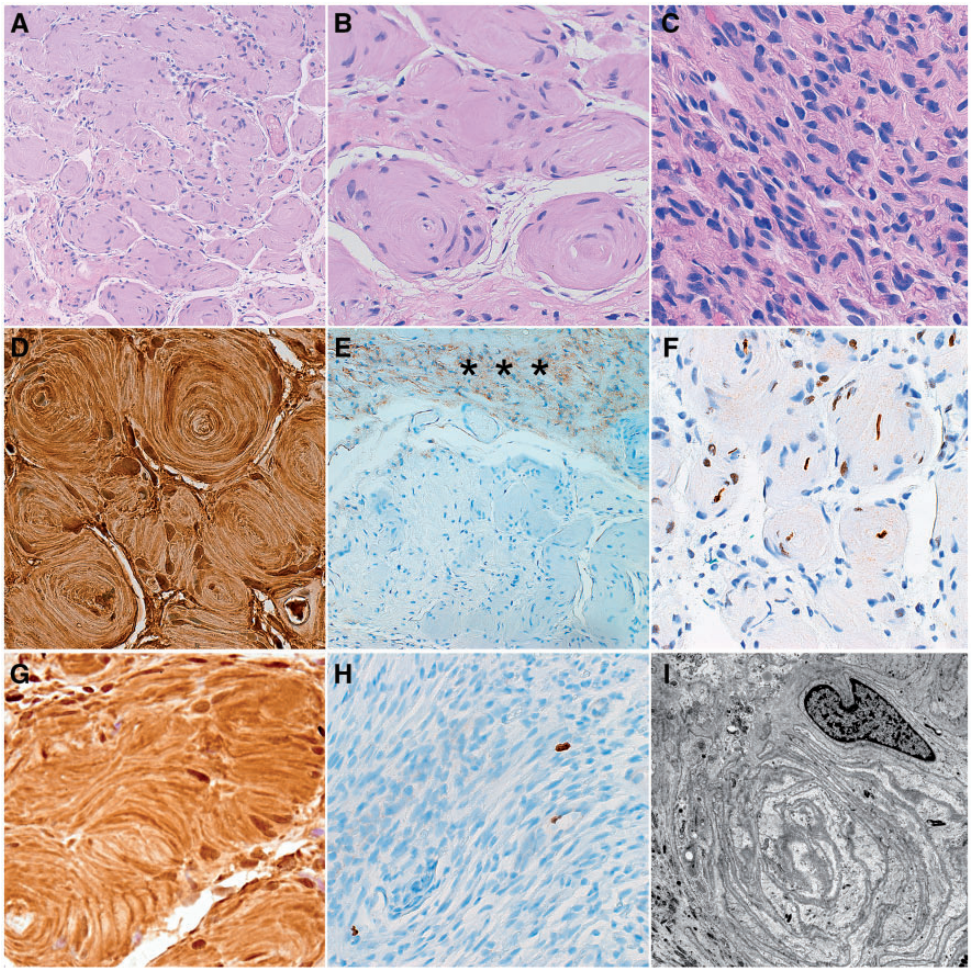

FIGURE 2. Pathologic features of localized hypertrophic neuropathy in RASopathy. Cross sections of the peripheral nerve fascicle demonstrated circular arrangements of Schwann cell processes around individual axons ("onion bulbs") **(A, B)**. Longitudinal sections showed an increase in endoneurial cellularity, but no mitotic activity **(C)**. Immunohistochemical stains demonstrated strong reactivity for S100 in onion bulbs **(D)**, while EMA immunoreactivity was limited to perineurium (asterisks) **(E)**. Neurofilament protein highlighted individual axons **(F)**. Strong pERK staining was detected in Schwann cells **(G)**. Ki67 labeling index was very low (<1%) **(H)**. Electron microscopy demonstrated concentric arrangements of Schwann cell processes **(I).**

RAS regulator, *SPRED1* (14). Interestingly, most of the RASopathies other than NF1 are not usually associated with the development of peripheral nerve sheath tumors, with very few exceptions. Soft tissue (15) and intraneural perineuriomas (16) may rarely develop in NF2 patients. There is a report of multiple spinal nerve or plexi enlargements in Noonan patients with *PTPN11* (17, 18) or *SOS1* mutations (19). One Noonan patient with a *PTPN11* mutation presented with a neuropathy and enlargement of the sacral plexus but no biopsy was performed (20). Another Noonan patient with a *PTPN11* mutation, identified in a study of 3 families, developed enlargements of multiple nerves and fascicular biopsy of the tibial nerve demonstrated onion bulb formation as well as a separate neurofibroma (17), changes similar to the case presented herein. Of interest to the present case, one patient with a germline *KRAS* mutation presented with enlargement of multiple nerve roots and plexi, and biopsy of a superficial nodule revealed a nerve sheath tumor interpreted as schwannoma (21).

Several features in our opinion help characterize this current lesion as neoplastic: It resembles intraneural perineurioma in all respects, a recognized clonal neoplasm of nerve, except that the lesional cells are Schwann cells; it is a mass-like lesion (on imaging favored to represent tumor) with an increase in cellularity attributable to Schwann cells, despite low level proliferation; and, finally, an oncogenic mutation involving a known oncogenic driver was identified in the lesional cells (but not in the germline). This was associated with strong phospho-ERK immunoreactivity of the peripheral nerve lesion, consistent with downstream MAPK pathway activation. The oncogenic mutation identified in the skin lesion raises an analogy to other neoplastic disorders of neural crest origin, such as neurocutaneous melanocytosis, where the skin and intracranial lesions, considered bonafide neoplasms, share the same oncogenic mutation leading to MAPK pathway activation, usually in the form of a *NRAS* mutation (in contrast to *KRAS* mutation in our case). The fascinating embryologic ori-

gin of these lesions appears to involve a postzygotic mutation in a melanocytic/Schwann cell precursor, with the timing of the mutation reflecting the precise phenotype observed (22). Migration of the precursor cell may be wide, since the lesions were bilateral in our case.

In conclusion, true LHN is a rare entity that may be associated with activating *KRAS* gene mutations, and therefore a subset may represent true neoplasms of Schwann cells developing in the context of a RASopathy. RASopathies are among the largest groups of genetic syndromes, and it is important to recognize them since targeted therapies are developing or undergoing clinical trials to potentially treat patients affected by this group of diseases.

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