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Multilineage *ACTB* mutation in a patient with fibro-osseous maxillary lesion and pilocytic astrocytoma

Young H. Lim[#1,2,3], **Andrea B. Burke**[#4,5], **Mary S. Roberts**[4], **Michael T. Collins**[4], and **Keith A. Choate**[1,2,3]

[1]Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut [2]Department of Pathology, Yale University School of Medicine, New Haven, Connecticut [3]Department of Genetics, Yale University School of Medicine, New Haven, Connecticut [4]Skeletal Disorders and Mineral Homeostasis Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland [5]Division of Oral and Maxillofacial Surgery, School of Dentistry, University of Washington, Seattle, Washington

[#] These authors contributed equally to this work.

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Somatic, de novo mutations affecting pluripotent cells and occurring early in embryogenesis can generate lesions in distinct germ layers (Hall, 1988; Youssoufian & Pyeritz, 2002). Such events give rise to syndromic mosaic disorders including Schimmelpenning-Feuerstein-Mims syndrome (SFM), Cutaneous Skeletal Hypophosphatemia syndrome, and McCune-Albright syndrome (MAS), wherein a multipotent cell acquires a postzygotic mutation in the Ras subfamily or GNAS, respectively, prior to its replication and differentiation into segments of mutant neural, endocrine, skeletal, and skin tissues (Groesser et al., 2012; Lim et al., 2014; Weinstein et al., 1991). Consequently, multiple end organs present a constellation of symptoms: SFM features ipsilateral keratinocytic or sebaceous nevi associated with central nervous system disorders including epilepsy, seizures, and mental retardation, as well as ocular, skeletal, cardiovascular, and genitourinary anomalies (Groesser et al., 2012), while MAS patients exhibit melanotic skin patches, polyostotic fibrous dysplasia of the bones, and endocrinopathies (Robinson, Collins, & Boyce, 2016; Weinstein et al., 1991). Unless the postzygotic mutation affects gonadal tissues as in germline (mutation present in gametes) or gonosomal (mutation in both soma and gametes)

Correspondence Keith A. Choate, Departments of Dermatology, Pathology, and Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06519. keith.choate@yale.edu; Michael T. Collins, Skeletal Disorders and Mineral Homeostasis Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive, Bethesda, MD 20892-4320. mc247k@nih.gov.

CONFLICT OF INTEREST

There is no conflict of interests.

SUPPORTING INFORMATION

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mosaicism, such disorders are not transmitted to subsequent generations (Happle, 2016). Certain mutations, like the AKT1 variants that cause the Proteus syndrome, only manifest as mosaic disorders, as they are likely constitutionally lethal (Lindhurst et al., 2011). Patterned distributions of lesional tissue such as unilateral or linear presentations suggest genetic mosaicism (Happle, 2016). However, exceptions such as the coat-like pattern of giant congenital melanocytic nevi arising most commonly from somatic activating mutation in neuroblastoma RAS viral oncogene homolog (NRAS), exist (Charbel et al., 2014).

Here, we report a 5-year-old boy (MOS100) who presented at 3 years of age with asymptomatic expansion of the right maxillary alveolar ridge (Figure 1a). His parents gave informed consent for him to be evaluated under a NIDCR IRB-approved protocol and a Yale Human Investigation committee-approved study protocol. Dental radiographic imaging and computed tomography (CT) revealed a fibro-osseous lesion suggestive of fibrous dysplasia (Figure 1b), but without “ground-glass” appearance. The primary teeth exhibited abnormal growth and one permanent tooth bud was missing. Endocrine workup was unremarkable, and a bone series did not indicate evidence of extragnathic fibro-osseous bone lesions. A 6-month follow-up CT and magnetic resonance imaging (MRI) revealed a slow expansion of the maxillary lesion, and incidental findings of a new brain stem mass with mild obstructive hydrocephalus (Figure 1c,d). Histopathologic assessment of the maxillary lesion demonstrated hypermineralized woven bone, with regions of dentinal tubules (Figure 2a,b) suggestive of a tooth cell origin. The patient underwent biopsy of brain stem lesion and placement of ventriculoperitoneal shunt, and histopathology was consistent with pilocytic astrocytoma (Figure 2c). The tumor was not mitotically active, with a mildly elevated Ki67 proliferation index (5%). Florescence in situ hybridization using the D7Z1 DNA Probe (chromosome 7α satellite DNA) at 7p11.1-q11.1 and homebrew probes RP11-767F15 and RP11-60F17 did not identify *BRAF* duplication at 7q34 in the brain lesion (Tian et al., 2011) and Sanger sequencing did not identify hotspot mutations in *BRAF* (V600) and *GNAS* (R201) mutation in the brain and maxillary lesions (data not shown).

Paired whole exome sequencing was performed using genomic DNA isolated from blood and biopsy of the maxillary lesion (Supporting Information Supplementary Methods), identifying a single somatic c.439C>T, p.147R>C mutation in beta-actin (*ACTB*). The mutation was confirmed via Sanger sequencing of DNA isolated from cells cultured from affected bone tissue (Supplementary Methods and Supporting Information Table 1). Suspecting that the subject’s astrocytoma resulted from widespread *ACTB* mosaicism, we performed targeted sequencing of *ACTB* in brain biopsy DNA. In so doing, we found the identical c.439C>T, p.147R>C *ACTB* mutation (Figure 2d), confirming multilineage *ACTB* mosaicism affecting the mesoderm (maxilla) and ectoderm (astrocytes) (Figure 2d).

Beta-actin is one of six isoforms of the highly conserved, ubiquitous “housekeeping” actin, a cytoskeletal protein involved in cell motility, adhesion, and embryonic development (Bunnell, Burbach, Shimizu, & Ervasti, 2011). Distinct germline mutations in *ACTB* including p.74G>S, p.117E>K, p.120T>I, and p.196R>H have been reported to cause Baraitser-Winter Cerebrofrontofacial syndrome (BWCFF) (Di Donato et al., 2014; Riviere et al., 2012), an autosomal dominant developmental disorder that features characteristic facies with hypertelorism, ptosis, broad nasal bridge, and pointed chin, along with mental disability

and structural brain abnormalities due presumably to impaired neuronal migration (Verloes, Drunat, Pilz, & Di Donato, 2015). Functional analyses of BWCFF *ACTB* mutations found altered cell adhesion and polymer instability (Johnston et al., 2013). Other germline *ACTB* mutations including p.183R>W cause juvenile-onset dystonia with hearing loss and developmental delay (Conboy et al., 2017), and this mutation led to alter depolymerization dynamics leading to a morphologically abnormal actin cytoskeleton (Procaccio et al., 2006).

Notably, the same postzygotic p.147R>C mutation in *ACTB* as in our subject was recently identified in a majority (14 of 23 examined, 61%) of Becker's nevi (BN) and Becker's nevus syndrome (BNS) subjects, along with a p.147R>S mutation. BN are common benign hamartomas affecting approximately 1 in 200 individuals, with syndromic cases having variable symptoms of cardiomyopathy, developmental delay, and unilateral breast hypoplasia (Cai et al., 2017). The mutation is localized to the pilar muscle of hair follicles, and was not shown to affect cytoskeletal actin organization or MAPK signaling. In mutation-expressing myoblasts treated with smoothened agonist, however, increased Gli1 expression was found, suggesting that the *ACTB* p.147R>C mutation in tissues leads to increased Hedgehog (Hh) signaling (Cai et al., 2017). In our case of fibro-osseous dysplasia with pilocytic astrocytoma, the mechanism by which the p.147R>C mutation gives rise to the neoplasms is unclear, though Gli-dependent aberrant activation of the Hh pathway is a feature of multiple solid tumors and affects tooth development (Hanna & Shevde, 2016; Hardcastle, Mo, Hui, & Sharpe, 1998). Moreover, the Hh pathway has been shown to regulate the growth of gliomas, and higher levels of GLI1 as well as PTCH, a transcriptional target of the Hh pathway, was demonstrated in pilocytic astrocytomas (Rush, Abel, Valadez, Pearson, & Cooper, 2010). Finally, *ACTB* has been demonstrated to play a role in the development of gliomas by facilitating interactions between heat shock proteins and 14-3-3 proteins, which are known to play a role in the patho-genesis of gliomas (Com et al., 2012). BNS can often feature skeletal defects, though they tend to be structural abnormalities such as hypoplasia of the shoulder girdle and extremities, scoliosis, and pectus excavatum (Happle & Koopman, 1997).

Our findings identify a novel clinical phenotype arising from multilineage *ACTB* mosaicism and extend the phenotypic spectrum of somatic *ACTB* mutation diseases to include a unique dental stem cell related facial skeletal disease and pilocytic astrocytoma. The presence of identical somatic mutation in two end organs arising from distinct germ layers confirms early postzygotic mutagenesis affecting a multipotent progenitor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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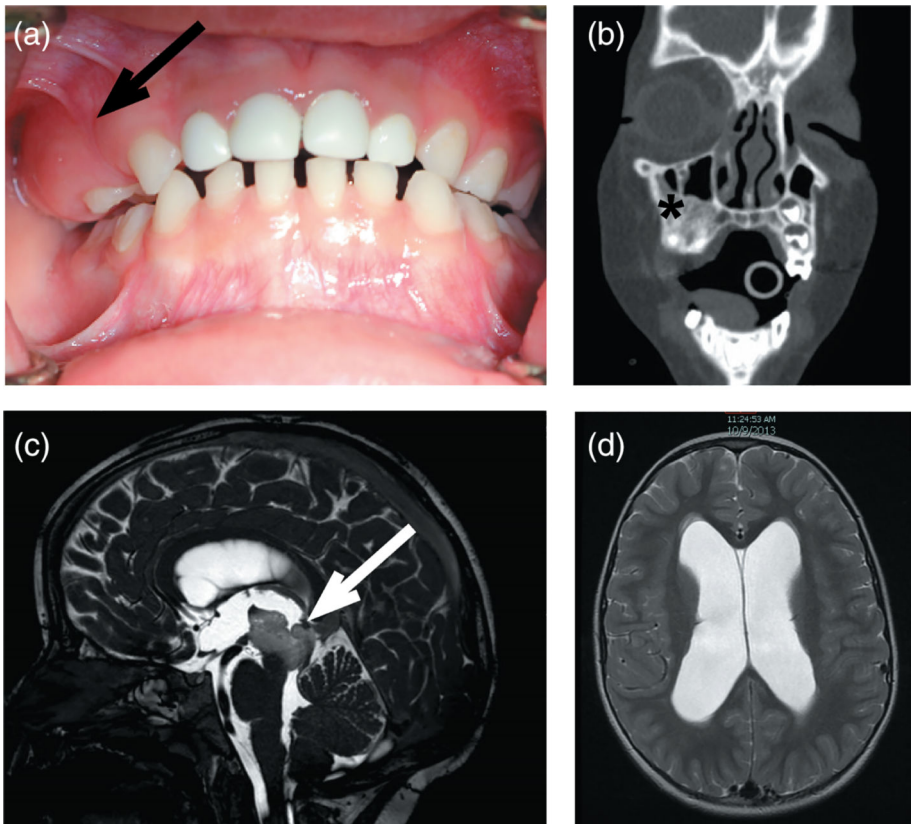

FIGURE 1.

Maxillary lesion and brain stem mass in MOS100. (a) Expansion of the maxillary bone was noted on routine dental examination (arrow). (b) Coronal CT demonstrated an area of dysplastic bone with a mostly sclerotic appearance (asterisk). (c) T2 sagittal MRI of the brain identified a central, soft tissue mass (arrow), which on biopsy was found to be a low-grade astrocytoma, and (d) associated hydrocephalus in the lateral ventricles [Color figure can be viewed at wileyonlinelibrary.com]

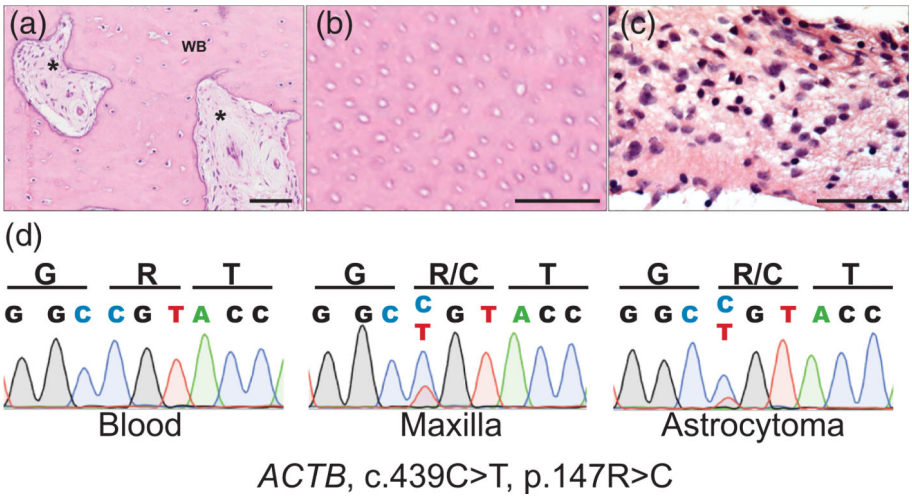

FIGURE 2.

ACTB mutation in maxillary lesion and pilocytic astrocytoma. (a) Representative 20× histology from an area of the maxillary lesion, which included areas of fibrosis (asterisk) within areas of woven bone (WB). (b) 40× view of tubular-like structures consistent with dentinal tubules. (c) 40× histology of smear of brain stem lesion demonstrates monomorphic population of glial tumor cells with elongated pilocytic processes in myxoid matrix, without Rosenthal fibers or eosinophilic granular bodies. (d) Sanger sequencing of *ACTB* demonstrates multilineage somatic c.439C>T, p.147R>C mutation, present in both the maxillary bone lesion (middle) and astrocytoma (right), which is absent in blood (left) [Color figure can be viewed at wileyonlinelibrary.com]