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A case report of mesenteric heterotopic ossification: Histopathologic and genetic findings

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ABSTRACT

Mesenteric heterotopic ossification (MHO) is very rare and occurs in mid- to late-adulthood, usually in the context of prior abdominal surgery. The mechanisms of MHO are unknown. Here we describe the case of a 72-year-old man with MHO. Standard histological staining revealed that MHO occurred through an endochondral process. By comparison to known mutations in genetic conditions of HO such as fibrodysplasia ossificans progressiva (FOP) and progressive osseous heteroplasia (POH), DNA sequencing analysis demonstrated the presence of a commonly occurring heterozygous synonymous polymorphism (c.690G>A; E230E) in the causative gene for FOP (*ACVR1/ALK2*). However, no frameshift, missense, or nonsense mutations in *ACVR1*, or in the causative gene for POH (*GNAS*), were found. Although genetic predisposition may play a role in MHO, our data suggest that mutations which occur in known hereditary conditions of HO are not the primary cause.

Contents

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1. Introduction

Heterotopic ossification (HO), or the formation of bone outside the normal skeleton, occurs in soft tissues, usually within muscular, adipose, or non-muscle fibrous/connective tissue [1, 2]. Genetic forms of HO, such as fibrodysplasia ossificans progressiva (FOP) and progressive osseous heteroplasia (POH), are usually clinically evident soon after birth and continue to advance throughout life [3, 4]. In contrast, nonhereditary HO (NHHO) tends to be limited and usually arises in the context of trauma, arthropathies, or age-related pathologies such as end-stage calcific valvular heart disease [1, 2].

HO is very rarely found in an intra-abdominal and specifically mesenteric location [5, 6]. Mesenteric HO (MHO) was described by Wilson et al. in 1999 in their evaluation of five intra-abdominal ossifying pseudotumors that were histologically identical to myositis ossificans [6]. MHO is an example of NHHO, usually occurs in mid- to late-adulthood, and shows a predilection for male patients [7, 8]. The great majority of patients present with bowel obstruction, and a history of prior abdominal surgery or trauma is almost always present [5–37]. Like most forms of NHHO, MHO may rarely recur, appears to occur in response to tissue injury or inflammation, and has no malignant potential [29]. However, MHO may, on occasion, contribute to patient mortality because of the effects of bowel obstruction in an already sick patient [29]. MHO occurs in the same types of soft connective tissue as genetic forms of HO.

The mechanism(s) of MHO are unknown, including relevant humoral factors, target cells, and genetic predisposition. Here we describe the case of a 72-year-old man with MHO, and investigate the histopathological as well as possible genetic influences on extraskeletal bone formation based on comparison to hereditary conditions in which ectopic ossification occurs.

2. Case history

The patient was a 72-year-old African American man with a history of hypertension, prostate cancer, and colon cancer who developed severe disabling bowel obstruction after a hemicolectomy for adenocarcinoma. The patient's resection for colon carcinoma was followed in a number of weeks by removal of keloid tissue from the abdomen. The patient died of massive pulmonary thromboembolism. At post-mortem examination, sectioning through the mesentery revealed dense fibrous tissue with calcified and ossified areas associated with adhesions and obstruction. The patient's skeleton and musculature were grossly normal and the joints were unremarkable. Malformations of the great toes (hallux valgus, malformed first metatarsal, and/or monophalangism), short malformed thumbs, brachydactyly, clinodactyly, and HO at locations outside of the abdomen were lacking. Except for focal areas of thickening in the tricuspid valve leaflets, mitral pulmonary and aortic valves were normal. Autopsy findings were otherwise unremarkable. No family members had a history of HO.

3. Materials and methods

3.1. Tissue processing and histology

Blood and paraffin-embedded mesenteric tissue with and without HO were obtained at autopsy. Archived samples of paraffin-embedded HO from patients with FOP and POH were obtained according to a protocol approved by the University of Pennsylvania Institutional Review Board and used as controls. Decalcified paraffin-embedded mesenteric tissue was sectioned into 6–8 μm slices. Sections were baked overnight in a dry incubator at 37 °C onto glass microscope slides. Tissue sections were deparaffinized with three 3 min immersions in xylenes at room temperature. Staining with Safranin O and Fast Green (counterstain) was performed by standard techniques.

3.2. ACVR1/GNAS mutation analysis

Genomic DNA was isolated from both blood and mesenteric tissue. Genomic DNA was isolated from blood using DNA blood-isolation reagents (QIAamp, Qiagen, Germantown, Maryland) according to the manufacturer's instructions. Genomic DNA was isolated from paraffin-embedded tissue using RecoverAll Total Nucleic Isolation Kit™ (Ambion, Austin, TX) according to the manufacturer's instructions.

Mutation analysis of the human *ACVR1* and *GNAS* genes was performed by polymerase chain reaction (PCR) amplification of genomic DNA (100 ng) using oligonucleotide primers flanking each of the protein coding exons [38, 39].

Analysis of *ACVR1* required the sequencing of nine exons. Two sets of primers were used for exon 3, dividing the exon into "3(a)" and "3(b)." For exons 2 (184 nucleotides (nt) PCR product size), 3(a) (449 nt), 4 (300 nt), and 6 (346 nt), PCR reaction volumes contained 2.0 μL Invitrogen System "10× PCR Buffer," 0.1 μL 20 mM dNTPs, 0.6 μL 50 mM MgCl2, 2.0 μL primer, 10.2 μL deionized Millipore™ purified water, and 0.1 μL of Taq Polymerase (Invitrogen; Waltham, Massachusetts). Exons 3(b) (438 nt PCR product size), 5 (350 bp), 7 (438 nt), and 8 (337 nt) contained buffers from the MasterAmp PCR Optimization (Epicentre, Madison, WI) buffer system. For exons 9 (299 nt PCR product size) and 10 (700 nt), PCR reactions were modified by using the Phusion High Fidelity Taq Polymerase (New England Biolabs, Inc., Ipswich, MA) and the Expand High FidelityPLUS Taq Polymerase (Roche Diagnostics Corporation, Indianapolis, IN), respectively. All exons analyzed contained 5 μL of genomic DNA at a 20 ng/μL concentration. Forward and reverse primers used for *ACVR1* sequencing have been previously reported [39].

Analysis of *GNAS* required the sequencing of 13 exons. Exons 1 (146 nt PCR product size) and 2 (162 nt) used the MasterAmp PCR Optimization buffer system (Epicentre, Madison, WI). PCR reaction volumes contained 10 μL MasterAmp PCR 2×F buffer, 1.2 μL primers, 3.68 μL deionized Millipore™ purified water, 5 μL genomic DNA (20 ng/μL concentration), and 0.12 μL Taq Polymerase (Invitrogen). For exons 3–13, 0.6 μL 50 mM MgCl2, 2 μL Invitrogen System "10× PCR Buffer," 0.1 μL 20 mM dNTPs, 10.2 μL deionized Millipore™ purified water, and 2.0 μL primers were used. Forward and reverse primers used for *GNAS* sequencing have been previously described [3, 38].

Amplified PCR products were electrophoresed through 1% agarose gels, stained with ethidium bromide (1 μg/mL), and purified using ExoSAP-IT for PCR Clean-Up (Affymetrix, Inc., Cleveland, OH) for exons 1–12 and QIAquick Gel Extraction (Qiagen) for exon 13. Eluted products were sequenced by the DNA Sequencing Core Facilities of the University of Pennsylvania.

DNA sequence polymorphisms were searched for presence in the National Center for Biotechnology Information (NCBI) database, release 49 (http://www.ncbi.nlm.nih.gov/RefSeq/) and the NCBI dbSNP database, build 136 (http://www.ncbi.nlm.nih.gov/projects/SNP/). Heterozygosity was reported according to the NCBI dbSNP database and the computation of average heterozygosity and standard error for dbSNP RefSNP clusters can be found at https://www.ncbi.nlm.nih.gov/SNP/Hetfreq.html.

4. Results

4.1. Histopathological analysis

Consecutive MHO tissue sections were analyzed to determine the general histological process for ossification. Fig. 1 shows that MHO occurred through endochondral ossification. Safranin O staining of MHO sections identified cartilage in close proximity to ectopic bone (Fig. 1A, left panel). HO as a result of FOP (Fig. 1A, right panel) also occurs through an endochondral process [4], while formation of HO in POH predominantly occurs through an intramembranous process (Fig. 1A, middle panel) [3].

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