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Genetic determinants of fibro-osseous lesions in aged inbred mice

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ABSTRACT

Fibro-osseous lesions in mice are progressive aging changes in which the bone marrow is replaced to various degrees by fibrovascular stroma and bony trabeculae in a wide variety of bones. The frequency and severity varied greatly among 28 different inbred mouse stains, predominantly affecting females, ranging from 0% for 10 strains to 100% for KK/HlJ and NZW/LacJ female mice. Few lesions were observed in male mice and for 23 of the strains, no lesions were observed in males for any of the cohorts. There were no significant correlations between strain-specific severities of fibro-osseous lesions and ovarian ($r = 0.11$; $P = 0.57$) or endometrial ($r = 0.03$; $P = 0.89$) cyst formation frequency or abnormalities in parathyroid glands. Frequency of fibro-osseous lesions was most strongly associated ($P < 10^{-6}$) with genome variations on chromosome (Chr) 8 at 90.6 and 90.8 Mb (rs33108071, rs33500669; $P = 5.0 \cdot 10^{-10}$, $1.3 \cdot 10^{-6}$), Chr 15 at 23.6 and 23.8 Mb (rs32087871, rs45770368; $P = 7.3 \cdot 10^{-7}$, $2.7 \cdot 10^{-6}$), and Chr 19 at 33.2, 33.4, and 33.6 Mb (rs311004232, rs30524929, rs30448815; $P = 2.8 \cdot 10^{-6}$, $2.8 \cdot 10^{-6}$, $2.8 \cdot 10^{-6}$) in genome-wide association studies (GWAS). The relatively large number of candidate genes identified in the GWAS analyses suggests that this may be an extremely complex polygenic disease. These results indicate that fibro-osseous lesions are surprisingly common in many inbred strains of laboratory mice as they age. While this presents little problem in most studies that utilize young animals, it may complicate aging studies, particularly those focused on bone.

1. Introduction

Idiopathic bone lesions are rare in inbred mice. Apart from inherited bone abnormalities (Elefteriou and Yang 2011; Woodward and Montgomery 1978), "cage kyphosis" (Sass et al., 1976; Sokoloff and Habermann 1958), and bone and cartilage tumors (Kavirayani and Foreman 2010; Kavirayani et al., 2012), fibro-osseous lesions of the bone develop primarily in the sternebrae, long bones, and vertebrae of female mice. Most commonly, those changes have been reported for B6C3F1 mice (i.e., F1 hybrid mice generated by crossing C57BL/6 females with C3H/HeJ male mice) (Gervais and Attia 2005; Sass and Montali 1980). Lesions can be observed in mice as young as 32 weeks (i.e., 4.5 months) of age. At 110 weeks (i.e., 16 months) of age the frequency of the lesions is 100% in female and less than 1% in male B6C3F1 hybrid mice (Albassam et al., 1991; Sass and Montali 1980). Although the histologic features of fibro-osseous lesions have been described in detail, the strains affected, the underlying genetic predisposition, and pathogenesis of the disease are still unclear.

The comparison between fibro-osseous lesions in mice and humans is difficult. Fibro-osseous lesions in mice are morphologically similar to fibrous osteodystrophy and myelofibrosis in humans and other species. However, osteodystrophy is associated with renal or parathyroid pathophysiology and mice with fibro-osseous lesions show no evidence of renal or parathyroid dysfunction. Also, mice with fibro-osseous lesions do not have associated myeloproliferation, a common observation with myelofibrosis. Thus, it has been suggested that fibro-osseous

Abbreviations: AB, Annerose Berndt; *Abcc6*, mouse ATP-binding cassette, sub-family C (CFTR/MRP), member 6 gene; CnSNPs, nonconsensus single nucleotide polymorphisms; CDK4, human cyclin-dependent kinase 4 protein; *Ephb4*, mouse ephrin receptor B4 gene; *FGF23*, human fibroblast growth factor 23 gene; *Gigyf1*, mouse GRB10 interacting GYF protein 1 gene; *GNAS*, human guanine nucleotide binding protein, alpha stimulating activity gene; IMPC, International Mouse Phenotyping Project; JMC, Justin M. Cates; JPS, John P. Sundberg; Mb, megabase; MDM2, murine double-minute type 2 protein; MoDIS, Mouse Disease Information System; MPD, Mouse Phenome Database; *Pilra*, mouse paired immunoglobin-like type 2 receptor alpha gene; PPH2, PolyPhen-2; PXE, pseudoxanthoma elasticum; SNPs, single nucleotide polymorphisms; *Zkscan1*, mouse zinc finger with KRAB and SCAN domains 1 gene..

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lesions observed in aging mice have a distinct pathogenesis. There is evidence for an associated hormonal imbalance, based on a strong sexual dichotomy where females are almost exclusively affected. Also, when mice of both sexes are treated with estrogen, they develop more severe fibro-osseous lesions earlier than seen in the spontaneous disease (Highman et al., 1981; Sass and Montali 1980; Silberberg and Silberberg 1970). Fibro-osseous lesions in B6C3F1 mice are often accompanied by ovarian cysts and cystic endometrial hyperplasia and, thus, could be caused by estrogen-producing cysts (Sass and Montali 1980). Misoprostol, an analog of prostaglandin E1, produces bone changes similar to those caused by estrogens in mice (Dodd and Port 1987). Lesions were also associated with increased plasma alkaline phosphatase levels in aged B6C3F1 hybrid female mice (Albassam et al. 1991).

The most common fibro-osseous lesions seen in human bone include fibrous dysplasia, ossifying fibroma (osteofibrous dysplasia), and central low-grade osteosarcoma. Molecular classification of fibro-osseous lesions can be useful in differentiating these bone lesions. For example, fibrous dysplasia is marked by a mutation in the alpha subunit of the G protein of guanine nucleotide binding protein, alpha stimulating (*GNAS*) (Liang et al., 2011). Other immunohistochemical markers such as cyclin-dependent kinase 4 protein (CDK4) and murine double-minute type 2 protein (MDM2) help to differentiate benign fibro-osseous lesions from low-grade osteosarcomas (Dujardin et al., 2011). Identifying the genetic basis of fibro-osseous lesions in the mouse provides the opportunity to compare these lesions on a molecular level and thereby to characterize novel model systems, which can be used to explore the human disease in greater depth.

This investigation aimed to identify the frequency and severity of fibro-osseous lesions of bone in 28 inbred and wild-derived mouse strains at various ages. Genome-wide scans were performed for female mice to identify the genetic variations associated with fibro-osseous lesions in this aging mouse population.

2. Materials and methods

2.1. Mice

The following 31 strains of inbred and wild-derived mice were used in a large-scale aging study (although only 28 strains survived to 20 months of age) (Sundberg et al., 2011; Yuan et al. 2009) and, as part of a detailed histopathological analysis, bones were examined for fibro-osseous lesions: 129S1/SvImJ, A/J, AKR/J, BALB/cByJ, BTBRT^{+} tf/J, BUB/BnJ, C3H/HeJ, C57BL/10J, C57BL/6J, C57BLKS/J, C57BR/cdJ, C57L/J, CAST/EiJ, CBA/J, DBA/2J, FVB/NJ, KK/HIJ, LP/J, MRL/MpJ, NOD.B10Sn-H2^{b}/J (NOD; a congenic strain with the NOD genetic background but with a histocompatibility locus from a diabetes-resistant strain), NON/ShiLtJ, NZO/HlLtJ, NZW/LacJ, P/J, PL/J, PWD/PhJ, RIIIS/J, SJL/J, SM/J, SWR/J, and WSB/J. Mice were part of a larger aging study by The Jackson Aging Center, which is described elsewhere (Sundberg et al., 2011). All mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6 to 8 weeks of age and sacrificed in cohorts at 12 and 20 months of age (cross-sectional study) or were allowed to age and were collected when moribund (longitudinal study). Mice were euthanized by CO_2 asphyxiation using methods approved by the American Veterinary Medical Association (Leary et al., 2013) and complete necropsies were performed (Silva and Sundberg 2012).

The mouse rooms were maintained on a 12 h light/12 h dark cycle and at an ambient temperature of 21–23 °C. Mice of the same gender (4 per cage) were housed in duplex polycarbonate cages (31 × 31 × 214 cm) on pressurized individually ventilated mouse racks (Thoran Caging System; Hazleton, PA) with a high efficiency particulate air-filtered supply and exhaust. Mice were allowed ad libitum access to acidified water (pH 2.8–3.2) and fed pellets containing 6% fat (LabDiet 5K52, PMI Nutritional International, Bentwood, MO). Regular monitoring for viruses, bacteria, parasites, and microsporidium showed that the colonies were free of any infestation (http://jaxmice.jax.org/geneticheath/index.html). All protocols were reviewed and approved by The Jackson Laboratory Animal Care and Use Committee (Animal Use Summary #07005).

2.2. Tissue fixation and preparation

Complete necropsies were performed at the time of euthanasia (Silva and Sundberg 2012). Bones (i.e., calvaria, shoulder and elbow with associated long bones, hip and knee with associated long bones, ribs, and vertebrae from the thoracic, lumbar, and coccygeal regions) were collected, fixed in Fekete's acid-alcohol-formalin overnight, and stored in 70% ethanol until processing. Bones were decalcified overnight in Cal-Ex (Fisher, Pittsburgh, PA) and briefly rinsed in water before trimming. Once the bones were trimmed and placed into the cassettes they were again rinsed in running water for a minimum of 4 h after which the tissues were processed routinely for histology, embedded in paraffin, cut into 6 μm sections, and stained with hematoxylin and eosin (H&E).

Additional serial sections were stained with Sirius Red and Mallory's trichrome stains for assessment of collagen deposition. Representative slides were subjected to immunohistochemistry for CD31 (Abcam cat# ab28364, Abcam, Cambridge, MA) as a marker of vascular endothelium (Ventana Medical Systems Discovery XT Automated Immunostainer, Oro Valley, AZ; http://tumor.informatics.jax.org/mtbwi/immunohistochemistry.jsp;jsessionid=747A7A0B4AE5B3AB58AF4CFDCDFE5496).

2.3. Histopathologic analysis

All tissue slides were reviewed by the same experienced, board certified veterinary pathologist (JPS) for histopathological analysis. Three strains (AKR/J, CAST/EiJ, and SJL/J) were not included in further analyses as they did not reach the age of 20 months (Sundberg et al., 2011). Fibro-osseous lesions were also evaluated by a board certified musculoskeletal pathologist (JMC) and compared to human archival clinical samples.

Prevalence of bone lesions was defined as the percentage of mice with diagnosed fibro-osseous lesions from the total number of mice per strain and gender (i.e., frequency). Lesions were also characterized by severity scores for each mouse (0 – normal; 1 – minimal; 2 – mild; 3 – moderate; 4 – severe). Average severity scores of all affected mice per strain and gender were calculated. Slides were reviewed and diagnoses were entered and coded by individual mouse using the Mouse Disease Information System (MoDIS) (Sundberg et al., 2009; Sundberg et al., 2008). Anatomical structures were defined using the Mouse Anatomy Ontology (MA) (Hayamizu et al., 2005) and disease diagnoses were entered using the Mouse Pathology ontology (MPATH) (Schofield et al., 2010a; Schofield et al., 2010b). Representative images of lesions in addition to those presented here are available on Pathbase (http://www.pathbase.net/) (Schofield et al., 2004a; Schofield et al., 2004b; Schofield et al. 2010b) and in the Mouse Tumor Biology Database (http://tumor.informatics.jax.org/) (Begley et al., 2014; Krupke et al., 2008).

2.4. Genome-wide association mapping

Genome-wide scans for frequency and severity of fibro-osseous lesions were performed using the expedited efficient mixed-model association (EMMAX) algorithm (Kang et al., 2010). Log-transformed strain frequencies of lesions in female mice were used as input data. The genome-wide scans were performed using four million (4 Mio. single nucleotide polymorphisms (SNP) by the NIEHS available for download at http://mouse.cs.ucla.edu/mousehapmap/full.html. Each SNP was evaluated individually and P-values were recorded as the strength of the genotype-phenotype associations.