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PERSPECTIVES

The Senile Osteoporosis Mouse Model SAMP-6: The Ideal Animal Model for Human Osteoporosis?

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Senile osteoporosis is characterized by increased susceptibility for low- trauma fracture. Because the condition is a major health problem of elderly individuals, it is essential to identify the underlying factors that determine bone strength and fracture risk.

Osteoporosis, however, is a slowly progressive disease, with its origins not only at the end of life, but also during growth. As a consequence, the disease is difficult to study in humans. Moreover, bone strength is a complex trait that is only partially explained by differences in bone mass or density. Other factors, such as bone turnover and geometrical and material properties, also contribute and even cause large variations in bone fragility among individuals with similar bone mass or density (1). These different factors influencing bone strength are not only determined by genes, but also by environmental factors (*e.g.*, calcium intake, physical activity, etc.) and their reciprocal interaction (2). The interplay between environmental factors and genotype is difficult to untangle and often confounds human genetic studies.

In order to circumvent these problems, animal models, like the mouse model, can be used. Mice provide an opportunity to keep the environment constant, are easy to handle, and also reproduce quickly. In addition, mouse models have a genome similar to humans that can easily be manipulated. Gene knock-out and inbred mice make it possible to focus on the function of one specific gene, as well as on the interaction of different genes (3;4). Unfortunately, mice, like other rodents, do not experience spontaneous fracture, which remains the hallmark of osteoporosis.

Senescence-accelerated mouse–prone 6 (SAMP6) was the first rodent model of senile (*i.e.*, type 2) osteoporosis with spontaneous fractures at older age. Most other "osteoporotic" animal models are selected on the basis of low bone mass and/or density, but do not experience spontaneous fracture. Manipulation of rodents also does not cause fracture. For instance, similar to postmenopausal osteoporosis in humans, sex steroid deficiency in gonadectomized rodents substantially reduces bone mass and changes bone architecture and turnover, but does not cause spontaneous fracture. SAMP6 therefore represents an exceptional model of bone fragility. In this perspective, we further discuss the relevance of the skeletal phenotype of SAMP6 mice.

Description of the SAMP6 Model

The SAMP6 mouse model was first described by Matsushita *et al*. (5) in the early 1980s. From the original AKR/J strain, the investigators used inbreeding to develop the senescence-accelerated mouse (SAM), consisting of nine prone (SAMP) and three resistant (SAMR) strains. Each SAMP strain has a relatively strain-specific pathologic phenotype and characteristics common to all SAMP strains (*i.e.*, accelerated senescence). Accelerated senescence may be caused by a higher generation of (and/or susceptibility to) oxidative stress (6). The different SAMP strains can be distinguished not only by phenotypical differences (they have diverse

geriatric disorders), but also by genetic and biochemical markers (7).

The parallel development of the different inbred strains derived from the same ancestor provides an opportunity to compare SAM mice. In most (but not all) studies, SAMP6 mice are compared with SAMR1 mice, which are resistant to osteoporosis and aging. When discussing the SAMP6 phenotype below, SAMR1 is used as a control, unless otherwise stated.

The SAMP6 mouse not only shows spontaneous tibial fracture at old age (20 months of age) (5), but also decreased bone strength, as confirmed by mechanical testing. Four-point bending tests show that at four months of age, SAMP6 femurs and tibias are already weaker and more brittle (8). Of interest, compression tests at the spine do not confirm decreased strength (9). Because such mechanical tests measure bone strength as a whole, a more precise look at SAMP6 bone properties is needed.

Age-related changes in bone mass are described in early reports of SAMP6 mice. SAMP6 mice show a significantly lower peak bone mass, compared with SAMR1 mice (5). Areal bone mineral density (aBMD) in the hindlimbs (5;8;10) is reduced. Similarly, trabecular bone volume in the spine and long bones of SAMP6 mice is also decreased because of reduced trabecular number and thickness (9;11-13). Because these differences are already apparent at maturity, low bone mass seems to result primarily from a deficiency in bone mass acquisition during growth and not from excessive bone loss during aging.

In the long bones of SAMP6 mice, low peak bone mass also seems to be related to increased periosteal width and medullary area, resulting in decreased cortical thickness (5;8;10). In contrast, vertebral bone dimensions are not different and are even smaller in SAMP6 mice at 12 months of age, because unlike SAMR1 mice, vertebral dimensions do not change with age (9).

Increasing size generally increases bone strength both in vertebrae and long bones. In the vertebrae, the pressure load is distributed on a larger bone area when size increases. SAMP6 vertebrae, however, do not show lower compression strength, even if their size is smaller, because cortical thickening can compensate for loss of bone area (9). In long bones, greater periosteal expansion increases the moment of inertia and should make bones more resistant to bending. Although the long bones of SAMP6 mice have improved moment of inertia, it is not reflected in increased strength. The discrepancy between bone geometry and biomechanical testing in long bones is unexpected and may point toward deficient material properties in SAMP6 mice that abrogate improved bone geometry.

There are several indications that SAMP6 bone material is more mineralized than SAMR1 bone. Although SAMP6 long bones have decreased aBMD, volumetric density, as determined by peripheral quantitative computed tomography (pQCT) and average cortical ash fraction *(i.e.,* ash weight/dry weight), is slightly increased (8). Additionally, nanoindentation measurements of material properties also show higher elastic modulus and hardness in SAMP6 mice, indicating a higher degree of mineralization (14). This nanoindentation modulus, however, is not confirmed by whole-bone bending. Although increased mineralization may contribute to the relative brittleness of SAMP6 bones, the influence of the higher mineral content observed in SAMP6 mice on bone strength is not clear. Increased mineralization alone seems not sufficient to explain decreased bone strength (8;14). Other compositional and structural features, such as increased porosity or impaired collagen structure, may also be important, but their roles still need to be determined (8;14).

Consistent with the increased inner perimeter of long bones, endosteal bone formation is decreased compared to SAMR1 (10;12;15;16). SAMP6 mice have fewer osteoblast progenitors and form less bone not only at the endocortical surface, but also at the trabecular bone surface. Because periosteal osteoblasts, in contrast with endocortical and cancellous osteoblasts, seem not to be insufficient in SAMP6 mice, factors in the bone marrow may be

responsible for the deficient activity and/or recruitment of endocortical and cancellous osteoblasts.

SAMP6 mice indeed exhibit several bone marrow abnormalities. Adipogenesis, for instance, is increased in SAMP6 mice (17). Because osteoblasts and adipocytes are of mesenchymal origin and stem from the same progenitor cells, the pathophysiology of increased adipogenesis and decreased osteoblastogenesis is probably related. The shift from osteoblasts toward more adipocytes may be attributed to decreased expression of interleukin 11 (IL-11) (17-19). In addition to the increase in adipocytes, myeloid cell numbers are also increased in the bone marrow of SAMP6 mice. Long-term bone marrow cultures of SAMP6 mice not only generate more myeloid progenitors, but also produce more IL-6 and colony-stimulating activity (17).

Besides intramedullary bone formation, bone resorption may also be lowered. At three to four months of age, osteoclast numbers in SAMP6 mice are decreased (12;18). In addition, bone resorption poorly responds to orchidectomy (13). In SAMR1 mice, orchidectomy causes an increase in the number of osteoblasts, bone formation rate, and osteoclast number. These changes are less or absent (no significant differences when small groups of mice are compared) when SAMP6 males are orchidectomized. Failure to upregulate osteoclastogenesis in SAMP6 mice is therefore probably the result of failure to increase osteoblast precursors after orchidectomy. As a result of decreased bone formation and resorption, SAMP6 mice exhibit decreased bone turnover at maturity.

Is SAMP6 a Model of Senile "Long Bone" Osteoporosis?

Bone fragility in elderly individuals may have its origin in growth and aging. Separating the structural abnormalities between growth and aging is not easy in humans, given the many years between attainment of peak bone mass and occurrence of osteoporotic fracture later in life. Mice have a shorter lifespan than do humans and can therefore be interesting to use as a model. The SAMP6 mouse model has many features that are reminiscent of senile osteoporosis in humans, including low aBMD, osteoblast insufficiency, and last but not least, spontaneous fracture later in life (20-22). For this reason, the SAMP6 mouse model provides an opportunity to study the pathogenesis of bone fragility.

In SAMP6 mice, there are several indications that at least a part of the bone fragility in the long bones finds its origin during growth. Already at 20 weeks of age, SAMP6 long bones have decreased aBMD, bone formation, and cortical thickness because of increased bone width (5;8;10;12). Therefore, it is necessary to take a closer look at the early bone changes and characteristics of SAMP6 mice.

Before puberty, at four weeks of age, aBMD seems not yet impaired (5;12;15). Chen *et al.* (15), however, showed that as early as eight weeks of age, trabecular bone in the distal femur of SAMP6 mice is already decreased. There are also several indications that although bone turnover is decreased at maturity in SAMP6 mice, it may be normal (or even increased) at an early age. An *in vitro* study showed that the number of osteoblast progenitors in bone marrow is not significantly different between SAMP6 and SAMR1 mice at the age of four weeks, but is decreased in SAMP6 mice of older age (12). Also, scanning electron microscopy images of the trabecular bone and endosteal surface of the femur of SAMP6 mice demonstrate decreased osteoblast number and ratio of forming/resorbing surfaces at five months of age, compared with no differences at one month of age (15). Furthermore, at five weeks of age, serum tartrate-resistant acid phosphatase and alkaline phosphatase levels in SAMP6 mice are increased (23). These data suggest that SAMP6 mice may have normal to increased bone turnover at an early age, in contrast to decreased bone turnover at maturity. In addition, bone formation and turnover seem only impaired at the endosteal (but not periosteal) site of long bones (10;16).

Decreased cortical thickness and larger perimeters also seem growth related and may already be present at four weeks of age (10;24). Although decreased cortical

thickness is not confirmed, there are indications that long bones of SAMP6 mice have enlarged width as early as four weeks of age (10;24). In humans, the femoral neck of the hip in female patients and their daughters is also enlarged and seems growth related (21;25). Therefore, wider bones in SAMP6 mice could be an early sign of bone fragility and not of bone strength. In the long term, wider bones can lead to more fragile bones, as aging makes bone perimeters increasingly greater and cortices thinner, resulting in unstable long bones that are more susceptible to buckling failure (8).

Compared with the SAMP6 model, other mouse models with different peak bone mass also show differences in the regulation of endosteal (but not periosteal) expansion (26;27). Genes that regulate endosteal bone formation may therefore be important for bone mass accrual and consequently determine bone strength. Recently, the role of periosteal bone formation not only during (but also after) growth with respect to the regulation of bone strength has received much attention (28). The SAMP6 model illustrates that endosteal bone turnover may also be an important determinant of future bone strength, especially during the early stages of bone growth.

In SAMP6 mice, bone fragility not only finds its origin in growth, but may also already be present before aging. Of interest, whole-bone bending tests already reveal decreased strength at maturity, although long bones of SAMP6 mice are wider and have a higher moment of inertia than SAMR1 bones. This finding, which may seem counterintuitive at first, can result from bone material failure, because bone strength is not only determined by bone geometry, but also by bone material and structure. Bone material is mainly composed of a hydroxyapatite-like mineral in a collagen matrix. Higher mineral content, as observed in SAMP6 mice, increases bone stiffness and the peak bone stress that a bone will tolerate, but also makes the bone more brittle and less tough. Higher collagen content, which has the opposite effect, makes bone more flexible and stronger (29;30). Although there are some indications that collagen content is decreased in SAMP6 mice, there is no other information about the properties of the collagen network (5;9). Porosity is also an important factor. In contrast with humans, mice do not have Haversian canals (the principal cause of porosity in human cortical bone), but they do show intracortical pores (31;32). However, the techniques often used to evaluate bone material properties, such as dual energy x-ray absorptiometry (DXA) and pQCT, cannot exclude a contribution by these pores in the cortex and this makes the real material density and properties unclear (33).

Decreased bone turnover and bone formation may further contribute to bone fragility in SAMP6 mice at maturity. Cortical bone turnover in rodents is mainly restricted to the periosteal and endosteal sites of the cortex. Lower turnover at endosteal sites can therefore lead to more mineralized bone. Higher mineralization may increase bone stiffness and thereby further weaken bone strength in this model. Even though there are some indications that SAMP6 mice might have increased mineralization, the material properties of SAMP6 bones remain largely unexplored and poorly understood.

Because SAMP6 mice already have decreased bone strength at maturity, and because a part of the bone fragility originates during growth, the term “senile osteoporosis” should be used with caution.

The SAMP6 Model and Vertebral Bone Strength

In contrast to the decreased bending strength of the tibia and femora of SAMP6 mice, compared with SAMR1 mice, compression testing of the caudal vertebrae shows no difference in strength between the two mouse strains (9). Although vertebral strength seems not impaired, SAMP6 vertebrae show similarities with humans with regard to spine fracture (21). Women with vertebral fracture have decreased vertebral size and volumetric BMD, compared with age-matched controls. Such decreased vertebral volumetric BMD was also already present in their daughters. Similarly, SAMP6 mice demonstrate decreased trabecular bone volume at four and 12 months of age

and decreased vertebral size at 12 months of age. The significance of this finding in the SAMP6 model, however, remains uncertain. Not only has fragility in the spine of SAMP6 mice not yet been determined, but also the use of a mouse model, a quadruped, for human spine osteoporosis may be less relevant.

A difference in strength between the long bones and vertebrae of SAMP6 mice is not unusual. Apart from the fact that their strength is measured by two different techniques (whole-bone bending and compression testing), long bones and vertebrae are designed for different functions. Humans at risk of hip fracture may or may not be less at risk of wrist or vertebral fracture (21;34;35). Different skeletal sites attain peak bone mass at different times and in different ways (*e.g.*, by increasing size, density, etc.) (27;36;37). In addition, the genetic regulation of bone strength is site and sex specific (26;35;37). Sexual dimorphism arising during growth is partly responsible for the difference in the number of osteoporotic fractures between men and women (36;38). In SAMP6 mice, a possible difference in peak bone mass acquisition between males and females and its effect on bone strength has not been evaluated.

Questions and Conclusions

SAMP6 mice again demonstrate that aBMD, as assessed by DXA, does not fully define underlying changes in structure and bone strength. In SAMP6 mice, lower aBMD is explained by cortical thinning, despite increased bone size and volumetric density. The use of aBMD as a discriminating factor in genetic studies comparing SAMP6 mice with a nonosteoporotic SAM subtype is therefore questionable. Thus far, genetic research has mainly focused on the genes involved in peak bone mass acquisition (39-42). However, bone strength, as illustrated by the SAMP6 model, is also determined by other factors, such as material properties and bone geometry, which have received less attention thus far.

Although SAMP6 is an interesting osteoporosis model, there are many open questions with respect to the pathophysiology of bone fragility in SAMP6 mice. It is not clear why the geometrical abnormalities in SAMP6 mice occur at an early age, whereas osteoblast insufficiency *in vitro* seems to occur later (*i.e.*, during growth). Many *in vitro* studies have focused on the age–related failure of osteoblasts to form new bone and support osteoclast formation in SAMP6 mice. Recent geometrical and mechanical studies of SAMP6 long bones, however, suggest that biomechanical failure is an early (not later) age-related feature in the mouse model.

Another important open question is the cause of osteoblast insufficiency in SAMP6 mice at the bone site in close contact with marrow, but not at the periosteum. This finding suggests that the bone marrow and its regulation of adipogenesis and osteoblastogenesis are very important determinants of bone strength in the SAMP6 model. There are also indications that fat deposition in bone marrow is increased in human osteoporosis (43). Whether fat deposition is also related to osteoblast insufficiency is not known.

Finally, it is not clear why both SAMP6 and human bones show regional differences in strength. Despite trabecular osteopenia and the absence of an age-related increase in size at the spine (two factors that should decrease bone strength), mechanical testing was not able to show weakness in this region. Also, in humans, decreased vertebral size and volumetric density are associated with spine fracture (27).

Although the extent to which osteoblastic insufficiency and structural and material abnormalities are related to bone strength is not yet fully understood, the SAMP6 model is a well-documented animal model with interesting features that are reminiscent of human osteoporosis. The SAMP6 model is therefore a valuable model to further identify the underlying mechanisms involved in bone strength and fracture risk.

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