INTRODUCTION. Bone fracture risk is currently mainly evaluated by bone mineral density (BMD) measurements. However, the architectural properties and organic components of the bone affect also the bone strength substantially. Del1(+/−) mice, heterozygous for a transgene harboring a 150 bp deletion mutation in Col2a1 gene for type II collagen, exhibit progressive osteoarthritis (1,2). Del1(+/+) mice, homozygous for the mutation develop severe chondrodysplasia, while in heterozygous Del11(+/−) mice the chondrodysplasia phenotype is milder; and have a delay in the appearance of primary ossification centers during spinal development (3,4). Abnormalities in formation of cancellous bone during fracture healing process with retarded chondrogenesis and osteogenesis was observed in 3-month-old mice (5). On the basis of these observations we decided to investigate the long-term consequences of type II collagen mutation in skeletally mature bone, and studied more closely the structural and biomechanical properties of long bones and spine in adult and aged Del1(+/−) mice.

METHODS. Twenty-five mice were killed and analyzed at age 15-21 months (Del1(+/-), n=9; control, n=16). The radiographic images of lower limbs were digitized, and the lengths of the tibiae and the length and thickness of femurs were measured. Peripheral quantitative computed tomography (pQCT) of femoral bone was also performed. The biomechanical properties of the femora were determined using the three-point-bending test (6). The maximal breaking force (F<sub>max</sub>) was defined as the bending load at failure. Stiffness was determined as the slope of the stress-displacement curve in the elastic region. Evaluation of the collagen organization of bone was carried out using quantitative polarized light microscopy (7). Hydroxyproline content was measured from humeri. Mid-sagittal sections of distal femora and cross-sections of femoral diaphysis of 15-month-old mice were immunostained with mouse monoclonal antibody E8 recognizing collagen type II by using EnVision™ staining kit. Two-tailed Mann-Whitney’s U-test was used to test statistical significance of the differences between experimental and control groups.

RESULTS. There was no difference in the length of the femoral bone. The cortical thickness, BMC, BMD of mid-diaphyseal femoral bone did not differ between the groups, neither did the hydroxyproline content of the humeri. The general morphology of femoral bone did not seem to be disturbed in Del1(+/−) mice. The maximal breaking force was 24% (p<0.01) lower in the 15-month-old transgenic female mice as compared with the age-matched controls (Fig. 1). The stiffness of femoral bone was 24% (p=0.057) lower in the transgenic mice as compared with the controls (Fig.1). The parallelism of the collagen network in transversal bone sections was 4.3% (p=0.017) lower in the transgenic mice as compared with control mice (Fig. 2). Also in the vertebral bone the birefringence was 27% (p=0.01) lower in the transgenic mice. Immunostaining for type II collagen was clearly noticed in the metaphyseal and diaphyseal femoral cortical bone of 15-month-old control and transgenic mice (Figs. 3B,C). The stained areas varied in size, and often acellular. However, no difference in the amount of these islets was observed between the transgenic and control groups.

DISCUSSION. Deletion mutation in collagen type II decreased the maximal breaking force of bone in the 15-month-old Del1(+/−) female mice as compared with the controls. A variety of analyses was performed to find out an explanation for the lower strength of femur.


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Figure 1. Breaking force (A) and stiffness (B) of femurs.

Figure 2. Parallelism of the collagen network measured from femoral midshaft cross-sections.

Figure 3. Polarization microscopy images of femoral cross-sections of control and transgenic Del1 (+/-) female mice showing the parallelism (0-100%) of the collagen network (A). Staining for type II collagen of distal femoral midsagittal section (B) showing articular cartilage (AC), growth plate (GP), and staining within the cortical bone (arrow). Cross-sections of the femoral diaphysis stained for type II collagen (C).