Genetic deletion of low-density lipoprotein receptor-related protein 5 increases cartilage degradation in instability-induced osteoarthritis

Liesbet Lodewyckx¹, Frank P. Luyten¹,² and Rik J. Lories¹,²

Abstract

Objective. The wingless-type MMTV integration site family (WNT) signalling pathway plays an important role in embryonic joint and bone development and has been associated with osteoporosis and osteoarthritis (OA). Loss-of-function mutations in low-density lipoprotein receptor-related protein 5 (LRP5), a WNT co-receptor, result in low bone mass. Lrp5⁻/⁻ mice also have low bone mass phenotypes. Recently an OA-susceptibility locus containing the LRP5 gene was suggested. We investigated the effects of loss of Lrp5 in joint biology in three different mouse models of OA.

Methods. Total body bone mineral parameters were measured by dual-energy X-ray absorptiometry. Trabecular and cortical bone parameters of tibia and femur were assessed ex vivo by peripheral quantitative CT. Osteoarthritic changes were induced in Lrp5⁻/⁻ and wild-type C57Bl/6J mice using the surgically induced destabilization of the medial meniscus model and the chemically induced papain and collagenase model. The severity of joint disease was investigated by histological analysis of the knee joints.

Results. Bone mineral density and weight were significantly decreased in Lrp5⁻/⁻ C57Bl/6J mice compared with their wild-type littermates. Surgically induced destabilization of the knee joint resulted in significantly increased cartilage degradation in the medial tibia of Lrp5⁻/⁻ mice compared with wild-type control mice. In the medial femur, a similar trend was found but did not reach statistical significance. In the papain- and collagenase-induced models, these differences were not observed. Inflammation scores were comparable between wild-type and Lrp5⁻/⁻ mice.

Conclusion. These data show that loss of function of Lrp5 increases cartilage degradation in mild instability-induced OA models in mice. Low bone mass density could have contributed to this effect.

Key words: WNT, wingless-type MMTV integration site family, LRP5, low-density lipoprotein receptor-related protein 5, OA, osteoporosis, bone, cartilage.

Introduction

Osteoarthritis (OA) is the most common chronic joint disorder, characterized by progressive damage in one or more joints, leading to loss of function, pain and disability. Genetic as well as acquired factors, such as obesity and excessive loading of the joints, play a role in the complex pathogenesis of the disease. Current evidence suggests that early events in the disease process can be recognized in both the articular cartilage and the subchondral bone [1], with progressive destruction of the cartilage and bone sclerosis. Cartilage damage develops when catabolic signals exceed anabolic or homeostatic efforts by the chondrocytes. Proteases break down the extracellular matrix molecules and the chondrocytes lose their differentiation status. Subchondral bone sclerosis is caused by increased local bone remodelling, with activation of the osteoblasts. Of interest, the sclerotic bone is often less mineralized, as the speed of calcification does not match the osteoid deposition by the osteoblasts. These typical features suggest that cartilage thickness and homeostasis as well as subchondral...
bone quality and architecture are important for joint integrity [2–5].

The wingless-type MMTV integration site family (WNT) signalling pathway plays an important role in embryonic joint and bone development and has been linked to both OA and osteoporosis [6]. WNTs are secreted growth factors that can induce their effects through different pathways. The canonical pathway is best studied [7]: when it is inactive, intracellular β-catenin is captured within a destruction complex, phosphorylated, ubiquitinated and degraded. On WNT ligand binding to a frizzled receptor and a low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6) co-receptor, β-catenin is released from the destruction complex, accumulates in the cytoplasm, translocates to the nucleus and activates a specific gene transcription profile.

LRP5 and 6 were identified as co-receptors for canonical WNT signalling [8]. Loss-of-function mutations in LRP5 lead to osteoporosis-pseudoglioma syndrome, an autosomal recessive disorder characterized by severe juvenile-onset osteoporosis with bone deformity and recurrent fractures and by persistent vitreal vasculature [9]. Dominant gain-of-function mutations of LRP5, on the other hand, cause high bone density disorders. Nevertheless, the direct role of LRP5 in bone metabolism has been controversial [10, 11].

Lrp5−/− mice have low bone mass phenotypes [12, 13]. Recently an OA-susceptibility locus was suggested on chromosome 11q12-13, which includes the LRP5 gene [14]. LRP5 is upregulated in articular cartilage from OA patients and linked to increased matrix metalloproteinase 13 expression in chondrocytes [15]. We therefore studied the development of OA in three different mouse models of the disease, comparing Lrp5−/− mice to wild-type animals.

Methods

Mice

C57Bl/6J male wild-type mice were from Janvier (Le Genest Saint Isle, France). Lrp5−/− founder mice in the Sv/129 background were a gift from Dr M. Warman (Harvard, Cambridge, MA, USA) [16] and were backcrossed to the C57Bl/6J background (more than five generations) in our animal facility. Heterozygous Lrp5+/− mice were used for mating to obtain male Lrp5−/− mice and wild-type littersmates used in the experiments. Genotyping was performed as described [16]. Mice were housed in conventional conditions: 12-h light/dark cycle, standard diet (1% calcium, 0.76% phosphate) and water ad libitum. All experiments were approved by the Ethical Committee for Animal Research, KU Leuven.

Arthritis models

Mild instability-induced OA was induced in the right knee of 8-week-old male C57Bl/6J Lrp5−/− or wild-type mice by surgical transection of the medial menisco-tibial ligament [destabilization of the medial meniscus (DMM) model] [5]. Sham-operated and contralateral knees (no operation) were used as control mice. Eight weeks after induction of the model, mice were sacrificed. Chemically OA was induced in 8-week-old male C57Bl/6J Lrp5−/− or wild-type mice by IA injection of 2 U/μl of type VII collagenase or 1% papain/0.03M l-cystein (all from Sigma-Aldrich, Bornem, Germany) in the right knee [5]. Contralateral knees injected with Dulbecco’s phosphate buffered saline (Lonza, Verviers, Belgium) were used as negative control mice. Mice were sacrificed 7 days later. Dissected knees of all models were processed for histology [5]. Five sections (100 μm apart) were stained with haematoxylin and eosin or Safranin O. Disease severity and the degree of articular cartilage degradation of all models was determined in a blinded way by two independent observers [17].

Lithium chloride treatment

Lithium chloride (LiCl) was orally administered to normalize bone density of Lrp5−/− mice. Eight-week-old male mice were gavage fed five times per week, with a daily dose of 200 or 400 mg/kg LiCl (Merck) in distilled water for 4 weeks. Control mice were fed with distilled water only. Four weeks after initiation of gavage feeding, the mice were sacrificed.

Assessment of bone parameters

Total body bone mineral density (BMD) (excluding the head) and bone mineral content (BMC) were measured in vivo by dual-energy X-ray absorptiometry (DEXA), using the PIXimus densitometer (Lunar Corp., Madison, WI, USA). Trabecular and cortical bone parameters of tibia and femur were assessed ex vivo by peripheral quantitative (pQ) CT with the XCT Research M system (Norland Medical Systems, Trumbull, CT, USA). Slices of thickness 0.2 mm were scanned with voxel size 0.07 mm. Three scans were taken 1.75–1.95 mm from the distal end of the femur or 1.5–1.7 mm from the proximal end of the tibia to determine trabecular bone parameters (trabecular density, content and area). An additional scan was taken 7 mm from the distal end of the femur or from the proximal end of the tibia (an area containing only cortical bone) to determine cortical bone parameters (cortical density, content, area and thickness and periosteal circumference). Density thresholds of 280 mg/cm³ and 710 mg/cm³ were used for trabecular and cortical bone, respectively.

Statistical analysis

Data are reported as mean (SEM) and were analysed by Mann–Whitney test using GraphPad Prism 5. P < 0.05 was considered significant.

Results

Lrp5−/− mice

Mating of heterozygous Lrp5+/− mice in the C57Bl/6J background produced litters with normal Mendelian ratios. Eight-week-old male Lrp5−/− mice had significantly lower weight [19.0 g (0.91 g)] compared with wild-type littersmates [23.38 g (0.68 g)] [P = 0.016 (n = 4–8)]. In vivo
measurement of bone parameters in 12-week-old mice showed significantly decreased total BMD and BMC in male Lrp5−/− mice compared with littermates (Table 1). Assessment of trabecular and cortical parameters ex vivo indicated significantly decreased trabecular and cortical BMD, cortical thickness (C. Th.) and area (C. Ar.) and periosteal circumference (PC) in femur or tibia of Lrp5−/− mice (Table 1). Gavage feeding with LiCl was not successful in normalizing bone parameters in Lrp5−/− mice in the C57Bl/6J background, in contrast to an earlier report using the Sv/129 background [Table 1 (data 200 mg/kg LiCl not shown)] [16]. This was a surprising finding, as LiCl treatment in wild-type C57Bl/6J mice was previously successful in increasing BMD [16].

OA induction in Lrp5−/− mice

We used three models that highlight specific aspects contributing to OA. Cartilage degradation was significantly increased in the medial tibia of Lrp5−/− mice 8 weeks after induction of the DMM model (Fig. 1A and D). A similar trend was observed in the medial femur, but it did not reach statistical significance (Fig. 1A and D). Cartilage degradation in sham-operated mice was nearly absent (data not shown). No differences between the two groups were observed after 7 days for the papain-(Fig. 1B and E) and collagenase-induced models (Fig. 1C and F). For all models, inflammation scores were comparable between Lrp5−/− and wild-type mice (data not shown).

Discussion

Lrp5−/− mice backcrossed into the C57Bl/6J inbred strain show increased severity of OA in the DMM model. This model is characterized by progressive development of OA-like lesions caused by mild instability and represents a clinically relevant model. In experiments in which the knee joints were severely challenged by papain or collagenase treatment, no differences were observed by the effect of LRP5 loss on OA. In these experiments, early time points were considered for the analysis, as the effect of papain fades and as collagenase injection leads to severe joint destruction in the long-term. Differences between the models lead us to hypothesize that the immediate impact of LRP5 deficiency is limited but that changes in the bony compartment of the joint make this genetic model more susceptible to a slowly progressive disease as seen in the DMM model.

Lrp5−/− mice have a low bone mass phenotype compared with wild-type littermates. Increased susceptibility to OA in an osteoporotic model may seem unexpected. The hypothesis of an inverse relationship between OA and osteoporosis [2] has been long-standing but controversial, as the molecular basis for some epidemiological observations has been elusive. WNTs play a role in cartilage and bone development and homeostasis, and this pathway seems to be of particular interest to provide such molecular insights. We demonstrated that mice deficient in WNT antagonist frizzled-related protein (Frzb) are more susceptible to cartilage damage in OA models [18]. This is

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**Table 1** Bone parameters of wild-type and Lrp5−/− mice measured by DEXA and pQCT at 12 weeks of age

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<thead>
<tr>
<th></th>
<th>Total body (P)</th>
<th>Tibia (P)</th>
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<th>Total body (P)</th>
<th>Tibia (P)</th>
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<tr>
<td>Wild-type</td>
<td>0.038 (0.002)</td>
<td>0.27 (0.012)</td>
<td>0.786 (0.016)</td>
<td>0.495 (0.028)</td>
<td>0.004 (0.013)</td>
<td>0.229 (0.012)</td>
</tr>
<tr>
<td>Lrp5−/−</td>
<td>0.024 (0.007)</td>
<td>0.23 (0.07)</td>
<td>0.018 (0.008)</td>
<td>0.018 (0.004)</td>
<td>0.004 (0.002)</td>
<td>0.325 (0.024)</td>
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Data are expressed as mean (S.E.M.); P-value by Mann-Whitney test for wild-type vs Lrp5−/− mice; n = 8 mice/group; ns: not significant; Tb: trabecular.
Fig. 1 Cartilage degradation in Lrp5−/− mice in models of OA.

(A) Cartilage degradation was increased in Lrp5−/− mice compared with wild-types in DMM models. No differences were observed in the chemically induced models (B and C) [*Mann–Whitney test: P-values are 0.012 (DMM medial tibia), 0.4 (DMM medial femur), P > 0.05 for papain and collagenase models; data are mean maximal histological score (S.E.M.); n = 8–16/group (DMM), 11 (papain), 9–10 (collagenase)]. (D and F) Frontal haematoxylin–safranin O-stained sections of wild-type and Lrp5−/− knees (medial) in the DMM (D), papain (E) and collagenase (F) models. Arrow: cartilage fibrillation; asterisk: loss of cartilage; open arrowhead: loss of proteoglycans.
partially explained by increased WNT-β-catenin signalling in the articular cartilage, resulting in upregulation of the tissue destructive enzyme MMP3. These observations seem to be in contrast with the data reported here. WNTs in the joint are tightly regulated, as both loss and gain of function models for β-catenin specifically restricted to the articular cartilage result in a spontaneous OA-like phenotype [19, 20].

Frzb−/− mice have increased cortical bone mass, density and thickness that likely result in changes in the impact of loading in the joint [18]. Their cortical bone is stiffer, and anabolic responses towards mechanical loading are greatly enhanced. These observations seem to be in contrast to the data reported here. Nonetheless, low BMD and disturbed bone architecture contribute to OA development as well. Low BMD was found to exacerbate cartilage breakdown in mice with a collagen type I mutation [4]. Mice carrying a mutation in growth differentiation factor 5 are susceptible to OA and have lower subchondral bone density and changes in the bone collagen fibres compared with wild-type animals [5]. In humans, subchondral bone volume increases during the sclerosis process, but mineralization is less effective because of enhanced bone turnover. Together, these data fit within the concept that the joint’s health is critically dependent on a molecular balance within the bone–cartilage biomechanical unit, with cartilage and bone homeostasis being tightly regulated processes involving signalling cascades such as WNTs.

The role of the LRP5 receptor in bone has been heavily debated [10, 11]. Yadav and colleagues [10] used tissue-specific genetic mouse models to show that LRP5 enhances bone formation not by direct effects on osteoblasts or osteocytes, but by inhibiting the expression in the duodenum of tryptophan hydroxylase 1, the rate-limiting enzyme in the serotonin biosynthetic pathway. Other authors provide compelling evidence for a direct effect on bone [11]. Our study does not allow us to define whether the effect of Lrp5 deletion is a direct effect on WNTs or secondary to serotonin produced in the gut. In the genetic model that we used, the LRP6 receptor is functional and WNT signalling is therefore probably reduced but not absent.

To correct for low bone mass, we tried to normalize BMD using LiCl before OA induction [16]. However, in contrast to the previous report, this was not successful. Differences in genetic background may affect responsiveness to LiCl. Strain-dependent differences are supported by the lack of effect on weight between the Lrp5−/− mice in the Sv129 background and their wild-type littermates, whereas weight was significantly distinct here in C57Bl/6J mice. Clément-Lacroix et al. increased BMD in wild-type C57Bl/6J mice. Although we adhered to the same protocol, calcium/phosphate content of the drinking water, vitamin D status of the mice and general health of the animals (including the presence of classical animal facility infections) may have had an impact. We focused on male mice, whereas the gender of mice analysed by Clément-Lacroix is not always clear. The C57Bl/6J mouse has low BMD and BMC, therefore further decreases because of LRP5 deficiency may be more difficult to correct as compared with bone parameters in wild-type mice.

In conclusion, induction of instability in the knee of Lrp5−/− mice increases cartilage degradation, further corroborating a critical role for WNT signalling in joint homeostasis. WNT signalling should be considered a therapeutic target but its complexity and regulation suggest that the development of such approaches remains a daunting task.

Rheumatology key messages

- Mild instability-induced OA in Lrp5−/− mice results in increased cartilage degradation.
- C57Bl/6J Lrp5−/− mice have low bone mass and are not responsive to LiCl treatment.

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