Published in final edited form as:

J Med Primatol. 2009 April ; 38(2): 97–106.

LRP5 Sequence and Polymorphisms in the Baboon

Alison F. Doubleday^{1,2,5}, Frederika A. Kaestle¹, Laura A. Cox^{3,4}, Shifra Birnbaum³, Michael C. Mahaney^{3,4}, and Lorena M. Havill³

¹Department of Anthropology, Indiana University, Bloomington, IN;

²Medical Sciences, Indiana University, Bloomington, IN;

³Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX;

⁴Southwest National Primate Research Center, San Antonio, TX

Abstract

Background—*LRP5* is known to have an important relationship with bone density and a variety of other biological processes. Mapping to human chromosome 11q13.2, *LRP5* shows considerable evolutionary conservation. Orthologs of this gene exist in many species, although comparison of human *LRP5* with other non-human primates has not been performed until now.

Methods—We report the complementary DNA (cDNA) sequence and deduced amino acid sequence for baboon *LRP5*, and compare the baboon and human sequences. cDNA sequences for 21 baboons are examined to identify single nucleotide polymorphisms (SNPs).

Results—Sequences of coding regions in human and baboon *LRP5* show 97% to 99% homology. Twenty-five SNPs are identified in the coding region of baboon *LRP5*.

Conclusions—The observed degree of coding sequence homology in *LRP5* leads us to expect that the baboon may serve as a useful model for future research into the role(s) of this gene in primate metabolic diseases.

Keywords

primate genetics; non-human primate model; Wnt signaling pathway; bone density

Introduction

Numerous studies of quantitative trait loci (QTLs) and single nucleotide polymorphisms (SNPs) in humans and mice demonstrate that low-density lipoprotein receptor-related protein 5 (*LRP5*), a member of the LDL receptor superfamily, plays an important role in several biological processes and that mutations in *LRP5* underlie a variety of inherited diseases. Mutations in the gene have been linked to Type I diabetes [12,41], hypercholesterolemia [13], atherosclerosis [33], Familial Exudative Vitreoretinopathy (FEVR) [22,35], and osteosarcoma [20], as well as several conditions involving variation in bone mineral density (BMD).

The genomic structure of *LRP5* is well-characterized in humans. The *LRP5* gene contains 23 exons and encodes a single-span transmembrane protein of 1,615 amino acid residues. The first 24 amino acids encode a signal peptide sequence. Four β -propeller domains separated by Epidermal Growth Factor-like (EGF) repeats are located immediately downstream of the

⁵Corresponding author: Medical Sciences, Jordan Hall 104, Indiana University 1001 E. Third St. Bloomington, IN 47405.
almfrec@indiana.edu Phone: (812) 360-2239, Fax: (812) 855-4436.

signal peptide sequence followed by three LDL-receptor type A (LA) domains, a single-pass transmembrane domain, and a cytoplasmic domain at the C-terminal end. Each of these regions has been characterized in some detail (In particular, see Springer, 1998; Hey, 1998; Rudenko et al, 2002; and Johnson and Summerfield, 2005). The four β - propeller domains contain six conserved YWTD (Tyr-Trp-Thr-Asp) repeats each. While YWTD is the common motif in these domains, structurally similar sequences such as LFAN and FFTN serve the same function [25]. The YWTD repeats form the blades of each β - propeller, which bring the EGF-like regions into close proximity to each other, and may play a role in ligand binding [25].

Considerable research interest in *LRP5* during the past ten years has been motivated largely by the results of whole genome linkage screens and candidate gene association studies that have strongly implicated the gene – and variants therein – in the regulation of bone mass and density [4,7,17,24,27-29,31,42,44]. Studies of the relationship between *LRP5* polymorphisms and skeletal phenotypes [2,14,22,28,32,43] reveal that mutations in *LRP5* in humans are responsible for skeletal disorders of both high and low BMD.

Due to the large number of people affected by bone fragility diseases (i.e. osteoporosis) the extent to which variation in the *LRP5* gene contributes to normal variation in BMD in the general human population is of considerable interest. Recent studies suggest that *LRP5* polymorphisms in humans may indeed contribute to population-level BMD variation [11,30,34,45]. Studies of inbred mice have provided important insight into the molecular mechanism of *LRP5* effects, but substantial differences in processes of skeletal maintenance and turnover between primate and non-primate species [3,21] make the development of a non-human primate model in which to study the role of *LRP5* in bone metabolism particularly relevant.

Genome-wide linkage screens using data from captive, pedigreed and genetically characterized baboons show a QTL affecting normal variation in forearm BMD in a region of the baboon chromosome 14 (PHA 14) that may be orthologous to the chromosomal region that harbors the *LRP5* gene in humans (HSA 11q13.2) [15]. Localization of bone-related QTLs to likely orthologous regions in both humans and baboons motivated us to further study and characterize PHA14 and its component genes in the latter species. Given the demonstrated relevance of one of these component genes, *LRP5*, to variation in bone mass and BMD in humans [2,4,11,14,22,24,28,30,32,34,42,43,45], we initiated a study to sequence this gene in baboons from the captive breeding population in which the PHA14 QTL was localized and genetic studies of age-related changes and pathology in bone and other organ systems are ongoing.

We report the results of a study with three main objectives. The first objective is to determine the DNA sequence of the coding regions of baboon *LRP5* and the predicted amino acid sequence of its protein product. The second, is to establish the degree of sequence similarity between humans and this potentially valuable non-human primate model species. The third, and last, objective of this study is to identify single nucleotide polymorphisms (SNPs) in the *LRP5* coding regions in the baboon.

Materials and Methods

The baboon model

The baboon shares physiological and developmental characteristics that make it particularly well-suited to studies of skeletal maintenance and turnover in humans. This animal shares with humans a relatively long lifespan (an important point because animals with shorter life spans do not maintain their skeletons in the same way humans do [21]). Further, baboons

and other Old World Monkeys have been shown to undergo bone loss with advancing age [1,10,18,26] as do humans. Baboon reproductive physiology and endocrinology are also quite similar to that of humans [3] in that the baboon experiences similar timing and phases of the menstrual cycle, parallels humans with regard to hormonal changes accompanying pregnancy [18], and undergoes a natural menopause [3,6,18]. These physiological similarities and the close genetic proximity between baboons and humans suggest that results of studies investigating baboon *LRP5* may be directly relevant to humans. Moreover, examination of specific *LRP5* domains across multiple species (i.e., mouse, baboon, and human) will likely reveal highly conserved regions of the gene that are potentially very important to skeletal maintenance.

Animal selection for *LRP5* resequencing and polymorphism identification

All animals from which data were obtained are part of the breeding colony at the Southwest National Primate Research Center (SNPRC)/Southwest Foundation for Biomedical Research (SFBR). The sample consists of olive baboons (*Papio hamadryas anubis*), yellow baboons (*Papio hamadryas cynocephalus*) and their hybrids. All procedures related to their treatment during their lives at SNPRC/SFBR were approved by the Institutional Animal Care and Use Committee in accordance with established guidelines. The current study involves isolation and sequencing of DNA from stored baboon liver specimens acquired from the SNPRC Blood, Tissue and DNA Repository. SNPRC archived baboon tissues were originally collected during routine necropsy. Animals were euthanized according to methods compliant with the AVMA panel on Euthanasia.

Tissue samples for this study are from a subset of the same group of animals in which a QTL affecting forearm BMD was detected near the location of the *LRP5* gene [15]. For these whole genome screens, animals with BMD data were organized into 11 large, extended pedigrees. To improve our chances of capturing all sequence variation in *LRP5* likely to be relevant to the previously localized BMD QTL, we used RT-PCR to amplify *LRP5* cDNA from 21 baboons (3 males, 18 females) discordant for BMD and representing ten pedigrees. All pedigree founders for which liver tissue was available were included. Additional animals were selected to ensure that one animal of relatively high BMD and one of relatively low BMD was represented in each pedigree (Table 2).

In order to characterize genetic diversity in baboon *LRP5*, SNPs were identified and examined for any resulting amino acid changes (non-synonymous). Animal ID number, sex, age, and forearm BMD z-scores for each individual are reported in Table 2. *LRP5* SNPs, exon location and resulting amino acid changes, if any, are listed in Table 3. Table 4 reports the alleles present at each location in individual baboons.

Resequencing and polymorphism identification for *LRP5* cDNA

Messenger RNA was extracted from baboon liver using the Ambion Trizol reagents and extraction protocol (Ambion Inc, Austin, TX). First strand cDNA synthesis used 2.5ug mRNA with Superscript III reverse transcriptase (Invitrogen Corporation, San Diego, CA). Complementary DNA for baboon *LRP5* was amplified in three overlapping fragments using rTaq polymerase according to manufacturer's instructions (Takara Shuzo Co., Kyoto, Japan). Primers for cDNA amplification were designed using Oligo software (Molecular Biology Insights, Inc.) and are listed in Table 1. Fragment one included *LRP5* exons 1-8. Fragment two included *LRP5* exons 7-18. Fragment three included exons 16-3'UTR.

PCR conditions for DNA amplification were: 1 min 30s denature at 96°C, followed by 35 cycles of 30s denature at 94°C, 30s annealing at 62°C and 5 min extension at 72°C with a final extension of 10 min at 72°C. Excess primers were eliminated using 5.3 units of shrimp

alkaline phosphatase (SAP), 27 units of exonuclease I (Exo I). The SAP/Exo I reaction was carried out at 37°C for 30 min followed by a 15-min heat inactivation at 80°C. The DNA from the SAP/Exo I reaction was used directly for DNA cycle sequencing [1min @ 96°C; 25 times (10sec @ 96°C; 4min @ 60°C)] using Big Dye Chemistry (v 3.1, Applied Biosystems, Inc., Foster City, CA) followed by analysis using an automated ABI DNA sequencer (Model 3730). The baboon cDNA sequence was aligned with human *LRP5* sequence (*Genbank ID: AP002366*) using Sequencher software (Gene Codes, Inc., Ann Arbor, MI) and Codon Code Aligner Software (CodonCode Corporation, Dedham, MA). Predicted protein structure was determined using ExPASy translate tool of the Swiss Institute of Bioinformatics (<https://www.expasy.ch/>). Protein alignment was conducted using ClustalW (www.ebi.ac.uk/clustalw/).

Results

cDNA and amino acid sequence alignment

Comparison of baboon and human *LRP5* cDNA nucleotide sequences shows a high level of conservation (97% identity). The partial baboon *LRP5* cDNA sequence can be found in the publicly accessible databank, GenBank (accession # FJ009119). Human sequence *AP002366*, used in this comparison, was obtained from GenBank.

Deduced amino acid sequence alignment between baboon and human shows a high degree of conservation with 99% amino acid identity. Figure 1 shows the baboon *LRP5* amino acid sequence aligned with the human sequence. The *LRP5* signal sequence, encoded by the first 24 amino acids, is not highly conserved and there is a difference in length between humans and baboons due to the deletion of three amino acids in baboon *LRP5*. Of 1,615 amino acids in *LRP5*, only four amino acid differences between human and baboon were identified, all of which are non-conserved: S315R, located in the first EGF-like domain; Y932H, located in the third EGF-like domain; and I1162V and I1191V, both located in the fourth β -propeller region. No differences were noted in the 5' region. The 3' region of baboon *LRP5* shares 94% identity with the human sequence and a CpG island, as well as three predicted miRNA sites in the 3' region, are conserved in the baboon.

Analysis of baboon *LRP5* cDNA sequence from 21 baboons discordant for BMD, showed 25 polymorphisms (Table 3). Two of these SNPs were nonsynonymous: G1469R and M1584L. Both of these are located in the cytoplasmic domain of *LRP5* and are not conserved.

Discussion

In spite of the high level of sequence homology exhibited between human and baboon *LRP5*, some differences warrant discussion. Two of the four amino acid differences identified between human and baboon *LRP5* are located in the epidermal growth factor-like (EGF) regions. Little is known about specific interactions of the *LRP5* EGF domains. Mice in which these regions are knocked out usually also show removal of the surrounding β -propeller domains, making it difficult to identify the independent effects of these two regions [24]. In humans, mutations in these domains, which are associated with particular bone density phenotypes, usually result in a *LRP5* truncated protein. This is not the case for the amino acid residues that differ between human and baboon where changes in the first and third EGF domains of baboon *LRP5* (S315R and Y932H, respectively) are predicted to have no impact on the protein structure. The amino acid changes in the fourth β -propeller region (Ile>Val) are also neutral changes and do not affect any of the conserved YWTD repeats located in the β -propeller region. Additionally, the conserved 5' and 3'UTR suggest that regulation of mRNA stability and translation are conserved. These results, predicting

functional conservation of *LRP5* between human and baboon, support the hypothesis that *LRP5* may play a similar role in the regulation of bone density in the baboon.

LRP5 baboon SNPs and amino acid changes

Identification of SNPs provides important information about gene sequence diversity and can be used to determine the relationship between genetic variation and phenotype. Twenty-five SNPs were identified in the coding regions of baboon *LRP5*.

The current human SNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/index.html>) records 18 coding-region SNPs for human *LRP5*. This is substantially fewer than the 25 SNPs detected in the coding region of the baboon gene, suggesting less variation in the human gene. Only two of the SNPs identified in baboon *LRP5* resulted in amino acid changes (G1469R and L1584M). Non-synonymous amino acid changes in human *LRP5*, identified in the current human SNP database, are located in exons 2 (rs41494349), 9 (rs4988321), 16 (rs11607268), 18 (rs3736228) and 22 (rs11574422). The two non-synonymous SNP amino acid changes identified in baboon *LRP5* are located in exons 21 and 23. These exons encode for the cytoplasmic portion of *LRP5*, as does exon 22, which contains human SNP rs11574422.

LRP5 mutations and BMD phenotypes

In general, previous research on human or mouse *LRP5* has shown that mutations leading to a phenotype of high BMD seem to be confined to the first β -propeller region. Most of these appear within or near the conserved YWTD repeats. Not all mutations found in this region, however, lead to increased BMD and mutations leading to a phenotype of low BMD have also been identified in the first β -propeller region [40,43]. Current research shows that mutations in the second, third and fourth β -propeller domains are primarily associated with osteoporosis pseudoglioma syndrome and FEVR conditions resulting in both BMD and vision problems [9,14,22,23,40]. FEVR has also been associated with mutations in the first and third LA domains. [9,22,40]. No non-synonymous SNPs were identified in these extracellular regions of baboon *LRP5*.

Both baboon *LRP5* residue changes are located in the cytoplasmic region of *LRP5* and may result in a change in protein function. The cytoplasmic portion of *LRP5* contains three PP(T/S)P motifs that bind axin [25]. All three repeats are conserved in the baboon *LRP5*, thus, axin binding should not be affected. However, one of the residues, (L1584M), lies in a region that acts as a binding site for FRAT1 [25], a protein that interacts with *LRP5* to inhibit phosphorylation of β -catenin by GSK-3, resulting in TCF1 transcriptional activation. Recent studies indicate that *LRP5*/FRAT1 interaction is required for activation of the canonical Wnt signaling pathway [16]. It is possible that a SNP located in this particular region of the cytoplasmic portion of *LRP5* may affect *LRP5*/FRAT1 interaction. If *LRP5*/FRAT1 interaction and subsequent β -catenin signaling are inhibited, the expected result is a low BMD phenotype due to reduced Wnt-signaling. Indeed, the SNP reported in this region (4789 C>A) was identified in several individuals with low BMD (Individuals 2, 4, 8, 10, 13, 14, 15, 16 and 17), however, it is also present in individuals exhibiting high BMD (individuals 1, 11, 12, and 19), and one individual with moderate BMD (Individual 9). Individuals homozygous for the C allele at this location (individuals 3, 5 and 7) also exhibit a wide range of BMD phenotypes, suggesting that the SNP is not strongly associated with any particular BMD phenotype in these animals. Interestingly, however, individuals exhibiting heterozygosity at this location all show relatively high BMD (individuals 6, 18, 20, 21). It is possible that a single copy of one of the alleles at this location (as exhibited by individuals 6, 18, 20, and 21) may create a conformational change in the protein leading to prolonged Wnt-signaling and, subsequently, higher BMD. The sample size presented here,

however, is too small to allow for statistical analyses to identify statistically significant differences. The current study is solely aimed at identifying SNPs for further examination, thus, the issue of specific SNPs and association with BMD phenotypes will require further investigation in a larger sample.

None of the SNPs discovered in baboon *LRP5* correspond to polymorphisms in human *LRP5* that are reported to cause disorders of high or low BMD. Bone density data, however, are available for the 21 subjects used in this study (Table 2) and specific care was taken to include individuals representing both relatively high and low bone density phenotypes. The next step in our investigations of *LRP5* is to genotype these SNPs in a larger sample, for which BMD data are available, in order to ascertain the relationship between bone phenotypes and sequence variation in the baboon. Although it is unlikely that the precise functional variants will match those in humans, it is highly likely that the exons or domains in which they occur and their mechanisms of action will correspond to the human condition.

Relevance of the study

Though rodent models have been used to generate and test hypotheses regarding the involvement of *LRP5* in bone maintenance, these animals are not ideal due to important differences in bone metabolism between rodents and humans [21]. The current study addresses the need for a well-characterized non-human primate model and provides evidence that the baboon is an appropriate animal model for studies of the role of this gene in primate skeletal maintenance, and in other metabolic processes in which *LRP5* plays a role.

The results seen here have general implications for conservation and homology between baboons and humans. We acknowledge that variation in a gene's expression, as well as the processing and modification of its transcripts and the translation of its products may be affected by DNA-level variation yet unmeasured by us in this study: e.g., introns, promoters, enhancers, miRNAs, epistatic genes, etc. However, we hypothesize that the observed degree of coding sequence homology between baboon and human *LRP5* also may be predictive of similar functions for this gene in these two species.

The *LRP5* sequence data generated by this study were obtained from analyses of DNA from the same pedigreed baboons in which genetics of bone metabolic disorders and disorders of other metabolic systems are ongoing, and which also contributed genetic data on which the baboon genome linkage map is based. Therefore, SNPs identified in this study have several immediate research applications. Not the least of these is using *LRP5* SNP genotypes to map *LRP5* to the genetic linkage map for PHA14. Doing so will improve resolution for one of the seven baboon chromosomes exhibiting rearrangements relative to their human orthologs [8,36]. Additionally, *LRP5* SNP genotypes can be used in pedigree-based association [15] and linkage analyses to test for effects of this gene on variation in BMD and a host of other bone-related phenotypes currently under study in these baboons.

Acknowledgments

Biomaterials collection for this study was supported in part by the following research grant from the U.S. National Institutes of Health (NIH): P01 HL028972. This investigation was conducted in part in facilities constructed with support from Research Facilities Improvement Program Grants C06 RR017515, C06 RR013556, C06 RR014578 from the National Center for research Resources (NCRR), NIH. The NIH NCRR base grant, P51 RR013986, supports the Southwest National Primate Research Center. The authors would like to thank the anonymous reviewers for helpful comments and suggestions.

This research was supported by a Southwest National Primate Research Center (SNPRC) Pilot Study Grant: 2 P51 RR013986-08. Biomaterials collection for this study was supported in part by the following research grant from the U.S. National Institutes of Health (NIH): P01 HL028972. This investigation was conducted in part in facilities

constructed with support from Research Facilities Improvement Program Grants C06 RR017515, C06 RR013556, C06 RR014578 from the National Center for research Resources (NCRR), NIH. The NIH NCRR base grant, P51 RR013986, supports the Southwest National Primate Research Center.

Literature Cited

1. Aufdemorte TB, Fox WC, Miller D, Buffum K, Holt GR, Carey KD. A non-human primate model for the study of osteoporosis and oral bone loss. *Bone*. 1993; 14:581–586. [PubMed: 8363909]
2. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med*. 2002; 346:1513–1521. [PubMed: 12015390]
3. Brommage R. Perspectives on using nonhuman primates to understand the etiology and treatment of postmenopausal osteoporosis. *Journal of Musculoskeletal Neuron Interaction*. 2001; 1:307–325.
4. Carn G, Koller DL, Peacock M, Hui SL, Evans WE, Conneally PM, Johnston CC Jr, Foroud T, Econs MJ. Sibling pair linkage and association studies between peak bone mineral density and the gene locus for the osteoclast-specific subunit (OC116) of the vacuolar proton pump on chromosome 11p12-13. *J Clin Endocrinol Metab*. 2002; 87:3819–3824. [PubMed: 12161516]
5. Cerroni AM, Tomlinson GA, Turnquist JE, Grynblas MD. Bone mineral density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. *Am J Phys Anthropol*. 2000; 113:389–410. [PubMed: 11042540]
6. Chen LD, Kushwaha RS, McGill HC Jr, Rice KS, Carey KD. Effect of naturally reduced ovarian function on plasma lipoprotein and 27-hydroxycholesterol levels in baboons (*Papio* sp.). *Atherosclerosis*. 1998; 136:89–98. [PubMed: 9544735]
7. Cohen Z, Kalichman L, Kobylansky E, Malkin I, Almog E, Livshits G. Cortical index and size of hand bones: segregation analysis and linkage with the 11q12-13 segment. *Med Sci Monit*. 2003; 9:MT13–20. [PubMed: 12640348]
8. Cox LA, Mahaney MC, Vandenberg JL, Rogers J. A second-generation genetic linkage map of the baboon (*Papio hamadryas*) genome. *Genomics*. 2006; 88:274–281. [PubMed: 16697552]
9. deCrecchio G, Simonelli F, Nunziata G, Mazzeo S, Greco G, Rinaldi E, Ventruto V, Ciccociola A, Miano M, Testa F, Curci A, D'Urso M, Rinaldi M, Cavaliere M, Castelluccio P. Autosomal recessive familial exudative vitreoretinopathy: evidence for genetic heterogeneity. *Clinical Genetics*. 1998; 54:315–320.
10. DeRousseau CJ. Aging in the musculoskeletal system of rhesus monkeys: III. Bone loss. *Am J Phys Anthropol*. 1985; 68:157–167. [PubMed: 4061606]
11. Ferrari SL, Deutsch S, Choudhury U, Chevalley T, Bonjour JP, Dermitzakis ET, Rizzoli R, Antonarakis SE. Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with variation in vertebral bone mass, vertebral bone size, and stature in whites. *Am J Hum Genet*. 2004; 74:866–875. [PubMed: 15077203]
12. Figueroa DJ, Hess JF, Ky B, Brown SD, Sandig V, Hermanowski-Vosatka A, Twells RC, Todd JA, Austin CP. Expression of the type I diabetes-associated gene LRP5 in macrophages, vitamin A system cells, and the Islets of Langerhans suggests multiple potential roles in diabetes. *J Histochem Cytochem*. 2000; 48:1357–1368. [PubMed: 10990489]
13. Fujino T, Asaba H, Kang MJ, Ikeda Y, Sone H, Takada S, Kim DH, Ioka RX, Ono M, Tomoyori H, Okubo M, Murase T, Kamataki A, Yamamoto J, Magoori K, Takahashi S, Miyamoto Y, Oishi H, Nose M, Okazaki M, Usui S, Imaizumi K, Yanagisawa M, Sakai J, Yamamoto TT. Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion. *Proc Natl Acad Sci U S A*. 2003; 100:229–234. [PubMed: 12509515]
14. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakis G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den

- Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell*. 2001; 107:513–523. [PubMed: 11719191]
15. Havill LM, Mahaney MC, Cox LA, Morin PA, Joslyn G, Rogers J. A quantitative trait locus for normal variation in forearm bone mineral density in pedigreed baboons maps to the ortholog of human chromosome 11q. *J Clin Endocrinol Metab*. 2005; 90:3638–3645. [PubMed: 15755864]
 16. Hay E, Faucheu C, Suc-Royer I, Touitou R, Stiot V, Vayssiere B, Baron R, Roman-Roman S, Rawadi G. Interaction between LRP5 and *Frat1* mediates the activation of the Wnt canonical pathway. *J Biol Chem*. 2005; 280:13616–13623. [PubMed: 15699046]
 17. Heaney C, Shalev H, Elbedour K, Carmi R, Staack JB, Sheffield VC, Beier DR. Human autosomal recessive osteopetrosis maps to 11q13, a position predicted by comparative mapping of the murine osteosclerosis (*oc*) mutation. *Hum Mol Genet*. 1998; 7:1407–1410. [PubMed: 9700194]
 18. Hendrickx, A.; Dukelow, W. Reproductive Biology. In: Henrickson, editor. *Nonhuman Primates in Biomedical Research: Biology and Management*. Academic Press; 1995. p. 147-191.
 19. Hey PJ, Twells RC, Phillips MS, Yusuke N, Brown SD, Kawaguchi Y, Cox R, Guochun X, Dugan V, Hammond H, Metzker ML, Todd JA, Hess JF. Cloning of a novel member of the low-density lipoprotein receptor family. *Gene*. 1998; 216:103–111. [PubMed: 9714764]
 20. Hoang BH, Kubo T, Healey JH, Sowers R, Mazza B, Yang R, Huvo AG, Meyers PA, Gorlick R. Expression of LDL receptor-related protein 5 (LRP5) as a novel marker for disease progression in high-grade osteosarcoma. *Int J Cancer*. 2004; 109:106–111. [PubMed: 14735475]
 21. Jerome CP, Peterson PE. Nonhuman primate models in skeletal research. *Bone*. 2001; 29:1–6. [PubMed: 11472884]
 22. Jiao X, Ventruito V, Trese MT, Shastry BS, Hejtmancik JF. Autosomal recessive familial exudative vitreoretinopathy is associated with mutations in LRP5. *Am J Hum Genet*. 2004; 75:878–884. [PubMed: 15346351]
 23. Jin L, Lau H, Smith D, Lau K, Cheung P, Kwan E, Low L, Chan V, Kung A. A family with osteoporosis-pseudoglioma syndrome (OPG) due to compound heterozygous mutation of the LRP5 gene. *Journal of Bone and Mineral Research*. 2004; 19:S129.
 24. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). *Am J Hum Genet*. 1997; 60:1326–1332. [PubMed: 9199553]
 25. Johnson ML, Summerfield DT. Parameters of LRP5 from a structural and molecular perspective. *Crit Rev Eukaryot Gene Expr*. 2005; 15:229–242. [PubMed: 16390319]
 26. Kammerer C, Sparks M, Rogers J. Effects of age, sex and heredity on measures of bone mass in baboons (*Papio Hamadryas*). *Journal of Medical Primatology*. 1995; 24:236–242. [PubMed: 8750499]
 27. Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES. Quantitative trait loci affecting peak bone mineral density in mice. *J Bone Miner Res*. 1998; 13:1648–1656. [PubMed: 9797472]
 28. Koay MA, Woon PY, Zhang Y, Miles LJ, Duncan EL, Ralston SH, Compston JE, Cooper C, Keen R, Langdahl BL, MacLelland A, O’Riordan J, Pols HA, Reid DM, Uitterlinden AG, Wass JA, Brown MA. Influence of LRP5 polymorphisms on normal variation in BMD. *J Bone Miner Res*. 2004; 19:1619–1627. [PubMed: 15355556]
 29. Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P, Curran ME, Rodriguez LA, Conneally PM, Joslyn G, Peacock M, Johnston CC, Foroud T. Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. *J Clin Endocrinol Metab*. 2000; 85:3116–3120. [PubMed: 10999795]
 30. Koller DL, Ichikawa S, Johnson ML, Lai D, Xuei X, Edenberg HJ, Conneally PM, Hui SL, Johnston CC, Peacock M, Foroud T, Econs MJ. Contribution of the LRP5 gene to normal variation in peak BMD in women. *J Bone Miner Res*. 2005; 20:75–80. [PubMed: 15619672]
 31. Koller DL, Rodriguez LA, Christian JC, Slemenda CW, Econs MJ, Hui SL, Morin P, Conneally PM, Joslyn G, Curran ME, Peacock M, Johnston CC, Foroud T. Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12-13. *J Bone Miner Res*. 1998; 13:1903–1908. [PubMed: 9844108]

32. Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am J Hum Genet.* 2002; 70:11–19. [PubMed: 11741193]
33. Magoori K, Kang MJ, Ito MR, Kakuuchi H, Ioka RX, Kamataki A, Kim DH, Asaba H, Iwasaki S, Takei YA, Sasaki M, Usui S, Okazaki M, Takahashi S, Ono M, Nose M, Sakai J, Fujino T, Yamamoto TT. Severe hypercholesterolemia, impaired fat tolerance, and advanced atherosclerosis in mice lacking both low density lipoprotein receptor-related protein 5 and apolipoprotein E. *J Biol Chem.* 2003; 278:11331–11336. [PubMed: 12509421]
34. Mitzuguchi TFI, Watanabe Y, Tsukamoto K, Tomita H, Tsujihata M, Ohta T, Kishino T, Matsumoto N, Minakami H, Niikawa N, Yoshiura KI. LRP5, low-density-lipoprotein-receptor-related protein 5, is a determinant for bone mineral density. *Japan Society of Human Genetics.* 2004; 49:80–86.
35. Qin M, Hayashi H, Oshima K, Tahira T, Hayashi K, Kondo H. Complexity of the genotype-phenotype correlation in familial exudative vitreoretinopathy with mutations in the LRP5 and/or FZD4 genes. *Hum Mutat.* 2005; 26:104–112. [PubMed: 15981244]
36. Rogers J, Mahaney MC, Witte SM, Nair S, Newman D, Wedel S, Rodriguez LA, Rice KS, Slifer SH, Perelygin A, Slifer M, Palladino-Negro P, Newman T, Chambers K, Joslyn G, Parry P, Morin PA. A genetic linkage map of the baboon (*Papio hamadryas*) genome based on human microsatellite polymorphisms. *Genomics.* 2000; 67:237–247. [PubMed: 10936045]
37. Rudenko G, Henry L, Henderson K, Ichtchenko K, Brown MS, Goldstein JL, Deisenhofer J. Structure of the LDL receptor extracellular domain at endosomal pH. *Science.* 2002; 298:2353–2358. [PubMed: 12459547]
38. Schweizer L, Varmus H. Wnt/Wingless signaling through beta-catenin requires the function of both LRP/Arrow and frizzled classes of receptors. *BMC Cell Biol.* 2003; 4:4. [PubMed: 12729465]
39. Springer TA. An extracellular beta-propeller module predicted in lipoprotein and scavenger receptors, tyrosine kinases, epidermal growth factor precursor, and extracellular matrix components. *J Mol Biol.* 1998; 283:837–862. [PubMed: 9790844]
40. Toomes C, Bottomley HM, Jackson RM, Towns KV, Scott S, Mackey DA, Craig JE, Jiang L, Yang Z, Trembath R, Woodruff G, Gregory-Evans CY, Gregory-Evans K, Parker MJ, Black GC, Downey LM, Zhang K, Inglehearn CF. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am J Hum Genet.* 2004; 74:721–730. [PubMed: 15024691]
41. Twells RC, Mein CA, Payne F, Veijola R, Gilbey M, Bright M, Timms A, Nakagawa Y, Snook H, Nutland S, Rance HE, Carr P, Dudbridge F, Cordell HJ, Cooper J, Tuomilehto-Wolf E, Tuomilehto J, Phillips M, Metzker M, Hess JF, Todd JA. Linkage and association mapping of the LRP5 locus on chromosome 11q13 in type 1 diabetes. *Hum Genet.* 2003; 113:99–105. [PubMed: 12700977]
42. Van Hul E, Gram J, Bollerslev J, Van Wesenbeeck L, Mathysen D, Andersen PE, Vanhoenacker F, Van Hul W. Localization of the gene causing autosomal dominant osteopetrosis type I to chromosome 11q12-13. *J Bone Miner Res.* 2002; 17:1111–1117. [PubMed: 12054167]
43. Van Wesenbeeck L, Cleiren E, Gram J, Beals R, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman M, de Vernejoul M, Bollerslev J, Van Hul W. Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. *American Journal of Human Genetics.* 2003; 72:763–771. [PubMed: 12579474]
44. Wynne F, Drummond FJ, Daly M, Brown M, Shanahan F, Molloy MG, Quane KA. Suggestive linkage of 2p22-25 and 11q12-13 with low bone mineral density at the lumbar spine in the Irish population. *Calcif Tissue Int.* 2003; 72:651–658. [PubMed: 14562992]

45. Zhang Z, Qin Y, He J, Huange Q, Li M, Hu Y, Liu Y. Association of polymorphisms in low-density lipoprotein receptor-related protein 5 gene with bone mineral density in postmenopausal Chinese women. *Acta Pharmacologica Sinica*. 2005; 26:1111–1116. [PubMed: 16115379]

Figure 1. Baboon amino acid sequence aligned with human sequence

The baboon sequence appears on the top line, the human sequence is on the bottom line. Residue numbers appear to the right of each line. Human sequence AP002366 was obtained from GenBank. Identical amino acids are indicated by *. : indicates non-synonymous mutations between human and baboon LRP5. The signal peptide sequence is enclosed in a box, β -propeller regions are underlined, EGF-like domains are in **bold**, LA repeat region is underlined in bold, the transmembrane domain is shaded in light gray and the cytoplasmic region is in italics.

Table 1
Primers used for PCR and sequencing of baboon Lrp5

cDNA was amplified in three fragments. Hum, bab, rh designate whether primer was designed from human, baboon, or rhesus.

PCR fragment		Primers
Exon 1-8	(F)humLRP5_04F:Ex01	GCCGGACAACATGGAGGCAG
	(R)humLRP5_05R:Ex08	CCCAGCAGCGTGAACCCGAAAA
Exon 7- 18	(F)humLRP5_03F:Ex07	GGGAGACGCCAAGACAGACAAG
	(R)rhLRP5_03R:Ex18	GCAGGCGCAGGTCCACACACT
Exon 16 - 3'UTR	(F)babLRP5_05F:Ex16	CGCCAACATTGTGCAGCCTCTG
	(R)babLRP5_02R:Ex23/3'utr	CCCGGCCAAGGTCAGGACGAGT

Fragment		Sequencing primers
Seq Exon 1-8 fragment	humLRP5_04F	GCCGGACAACATGGAGGCAG
	humLRP5_02F	CGGCGGAGTCAAGCTGGAGT
	humLRP5_08R	CACCTTCCGGGATGTGCCATTGAG
	humLRP5_09F	CGGATTGAGCGGCAGGGAT
	humLRP5_10F	GCGGCAGCCTTTCTTCCACA
	humLRP5_11R	TCCGTAGGTCCGTCCGCC
	humLRP5_12F	CCGCAAGATCCTGGTGTC
	humLRP5_05R	CCCAGCAGCGTGAACCCGAAAA
Seq Exon 7-18 fragment	humLRP5_03F	GGGAGACGCCAAGACAGACAAG
	humLRP5_07F	GGCCTGGAGCTGCTGAGTGACA
	humLRP5_06R	CGGGATGGCCACGTCGTGTGTA
	humLRP5_13F	GCCGACACTGGGACCAATAG
	humLRP5_14R	TCACGACCCGCTCCTGAC
	humLRP5_15F	AGCCCGCCCACCACCTTCTT
	humLRP5_16R	TGGGTCCCGTCGTCCTTG
	humLRP5_17F	GCCCTGGTGGTGGACAACAC
	babLRP5_05F	CGCCAACATTGTGCAGCCTCTG
	babLRP5_04R	GCACATGGGTGGGCTGAGAACT
Seq Exon 16-3'UTR fragment	rhLRP5_03R	GCAGGCGCAGGTCCACACACT
	babLRP5_05F	CGCCAACATTGTGCAGCCTCTG
	babLRP5_08F	ATCGACCGCCAGCAGCAGATGA
	babLRP5_04R	GCACATGGGTGGGCTGAGAACT
	rhLRP5_03R	GCAGGCGCAGGTCCACACACT
	rhLRP5_05F	CCATCTGCCTGCCAACCA
	babLRP5_10R	GATGATGCCAATGACGGG
	rhLRP5_04F	CCCTTCCCGCACGAGTATGTCA
	babLRP5_03R	CGAATGATGTAGGCGCTGTACGGT
	babLRP5_01R	GGGCGGAAGAGGTGGAAGTAG
	babLRP5_02R	CCCGGCCAAGGTCAGGACGAGT

Table 2
Individual baboon forearm BMD Z-scores

BMD (g/cm^2) was assessed in a previous study via dual energy x-ray absorptiometry (DXA) (Lunar DPX +6529, Lunar Corp., Madison, WI, U.S.A.). Z-scores for forearm BMD site are listed below. RadShf refers to diaphyseal radius at a point 33% proximal to the styloid process, BMD measurements and procedures are discussed in detail in Havill et al 2005.

Animal ID	SEX	AGE (years)	RadShf (Z-score)
1	F	27.8	1.45565
2	F	24.6	-0.24095
3	F	23.8	0.87726
4	F	25.8	-0.67795
5	F	24.9	-1.15351
6	F	23.4	-0.20239
7	F	23.9	-1.98895
8	F	22.8	-1.17921
9	F	23.9	-0.60083
10	F	24	-1.47483
11	F	23.8	1.42994
12	M	23.8	1.60988
13	F	20.8	-1.07639
14	F	21.1	-1.41057
15	F	24.1	-1.50054
16	F	26.8	-1.07639
17	F	20.3	-1.42342
18	F	21.2	0.06753
19	M	20.6	2.124
20	M	18.7	1.95692
21	F	16.1	1.77697

Table 3**Baboon LRP5 SNPs**

SNPs identified from comparison of cDNA sequence for 21 baboons, LRP5 exon location and any associated amino acid changes are reported.

– indicates no amino acid change. SNP numbering is derived from cDNA sequence and based off the first base encoding the transcription start site.

SNP	ALLELE 1	ALLELE 2	EXON	AMINO ACID CHANGE
354	C	T	2	-
369	C	T	2	-
507	C	T	2	-
576	G	A	3	-
951	C	T	5	-
1014	G	A	5	-
1116	G	T	6	-
1173	C	T	6	-
1215	C	T	6	-
1443	C	T	6	-
1665	G	A	8	-
1866	T	C	9	-
2088	T	C	9	-
2577	G	A	12	-
2862	C	T	12	-
3237	T	C	14	-
3258	C	T	14	-
3414	G	A	15	-
3621	C	T	16	-
3787	C	T	17	-
4284	C	T	20	-
4444	G	A	21	GLY>ARG
4452	C	G	21	-
4789	C	A	23	LEU>MET
4869	C	A	23	-

Table 4

Baboon LRP5 Alleles at SNP locations

Alleles at SNP locations identified from examination of cDNA sequence for 21 baboons. Individual identification numbers are in the far left column. The least common allele at each SNP site is in bold. N indicates an unknown allele. Animal ID numbers are listed in the far left column. SNP numbers are listed across the top of the table. SNP numbering is derived from cDNA sequence and based off the first base encoding the transcription start site.

Animal ID	354	369	507	576	951	1014	1116	1173	1215	1443	1665	1866	2088	2577	2862	3237	3258	3414	3621	3787	4284	4444	4452	4789	4869
1	CC	CT	CC	GG	CC	GG	GG	CC	CC	NN	GG	TT	TT	GG	CC	CT	CC	GG	CC	CT	CC	GG	CG	AA	AC
2	CC	CC	CC	AA	TT	GG	GG	NN	CC	CC	GG	CT	TT	GA	TT	TT	CC	GA	CT	CT	CC	GG	CC	AA	CC
3	CC	CT	CC	GG	CC	GG	GG	CC	CC	CC	GG	NN	TT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	CC	CC
4	CC	CC	CC	GG	CC	GA	GG	CC	CC	CC	GG	TT	TT	GG	CT	CT	CC	GG	CC	CC	CC	GG	CC	AA	CC
5	CC	TT	CC	GG	CC	GG	GG	CC	CC	CT	GG	TT	TT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	CC	CC
6	CC	CT	CC	GG	CC	GG	GG	CC	CC	CT	GG	TT	CT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	AC	CC
7	CC	CC	CC	GG	CC	GG	GG	CC	CC	CT	GG	TT	TT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	CC	CC
8	CC	CC	CC	GA	TT	GG	GG	CC	CC	CT	GG	TT	TT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	AA	CC
9	CC	CC	CT	AA	TT	GG	GG	CC	CT	CT	GG	TT	TT	GG	CC	CC	CC	GG	CC	CC	CT	GG	CC	AA	CC
10	CC	CC	CC	GG	CC	GG	GG	CC	CC	NN	NN	TT	TT	GG	CC	CT	CC	GG	CC	CC	GG	CC	CC	AA	CC
11	CC	CC	CC	GG	CT	GG	GG	CC	CC	CC	GG	TT	TT	GG	CT	TT	CC	GG	CC	CC	CC	GG	CC	AA	CC
12	CT	CT	CC	GG	CC	GG	GG	CC	CC	CC	GG	TT	TT	GG	CC	CT	CC	GG	CC	CC	CT	GG	CC	AA	CC
13	CC	CC	CC	GG	CT	GG	GG	CC	CC	CT	GA	TT	CT	GG	CT	TT	CT	GG	CC	CC	CC	GG	CC	AA	CC
14	CC	CT	CC	GG	CT	GG	GG	CC	CC	NN	GG	TT	TT	GG	CC	CC	CC	GG	CC	CC	CC	GG	GG	AA	AA
15	CC	CC	CC	GG	CT	GG	GG	CC	CC	NN	NN	TT	TT	GG	CT	TT	NN	GG	CC	CC	CC	GG	CC	AA	CC
16	CC	CC	CC	GA	CT	GG	TT	CT	CC	NN	GG	TT	TT	GG	CT	TT	CC	GG	CC	CC	CC	GG	CC	AA	CC
17	CT	CC	CC	GA	CT	GG	GG	CC	CC	CT	GG	TT	TT	GG	CC	CC	CC	GG	CC	CC	TT	GG	CC	AA	CC
18	CC	CC	CT	AA	TT	GG	GG	CC	CT	CC	GG	TT	TT	GG	TT	TT	CC	GG	CC	CC	CC	GG	CC	AC	CC
19	CC	CC	CC	GG	CC	GG	GG	CC	CC	CC	NN	TT	TT	GG	CC	NN	NN	GG	CC	CC	CC	GG	CC	AA	CC
20	CC	CT	CC	GA	CT	GG	GG	CC	CC	CC	GG	TT	TT	GG	TT	TT	NN	NN	CC	CC	CC	GG	CC	AC	CC
21	CC	CC	CC	GG	CT	GG	GG	CC	CC	CT	GG	NN	TT	GG	TT	TT	NN	GG	CC	CC	GA	CC	CC	AC	CC