Genetics of Osteoporosis - Studies on bone size, structure and strength in the rat

Lagerholm, Sofia

2010

Department of Clinical Sciences, Malmö
Clinical and Molecular Osteoporosis Unit

Genetics of Osteoporosis

Studies on Bone Size, Structure and Strength in the Rat

Sofia Lagerholm

LUND
UNIVERSITY
Faculty of Medicine

Academic Dissertation
With the permission of the Medical Faculty of Lund University, to be presented for public examination in Lecture Hall of Orthopedics, Entrance 21, 5th floor, Malmö University Hospital on April 29, 2010 at 1 p.m.

Faculty Opponent
Åsa Andersson, PhD, Associate Prof.
Faculty of Pharmaceutical Sciences, Copenhagen University

Main Supervisor: Prof. M.D Kristina Åkesson
Co-Supervisors: Prof. Holger Luthman and Maria Swanberg PhD
Department of Clinical Sciences, Malmö

Organization
LUND UNIVERSITY

Department of Clinical Sciences, Malmö
Clinical and Molecular Osteoporosis Unit
University Hospital MAS
Malmö, Sweden

Author(s)
Sofia Lagerholm

Document name
DOCTORAL DISSERTATION

Date of issue
April 29, 2010

Sponsoring organization

Title and subtitle

Genetics of Osteoporosis - Studies on Bone Size, Structure and Strength in the Rat

Abstract

Osteoporosis is characterized by decreased bone mineral density (BMD) leading to reduced bone strength and increased fracture risk. Several heritable components affect a bone's ability to resist fracture, including size, structure and strength; therefore identification of the genes underlying several bone characteristics will help elucidate the pathogenesis of fracture risk. Dissection of the genetic determinants of osteoporosis has been more effective in animal models than in human populations due to the possibility of environmental control and minimization of genetic heterogeneity.

The aim of this thesis was to identify quantitative trait loci (QTLs) affecting osteoporosis-related phenotypes in an F2 intercross between diabetic GK and non-diabetic F344 rats, differing in their mitochondrial (mt) DNA. For bone measurements, tibia were characterized using four different methods generating several skeletal determinants of fracture risk. Comprehensive analysis identified several chromosomal regions linked to bone size, structure and strength that were influenced by both sex- and reciprocal cross. A region on chromosome 1 was identified with linkage to several bone phenotypes and also fasting glucose, making this region as a strong candidate for the localisation of genes contributing to bone regulation and potentially type-2 diabetes. The observed interaction between nuclear QTLs for bone phenotypes and reciprocal cross, demonstrates a new interesting aspect when interpreting the genetics of phenotypes related to bone strength.

Furthermore, preliminary studies suggest that this rat model can be a useful tool to delineate the genetics of type-2 diabetes and osteoporosis in conjunction with lifestyle factors such as high-fat diet.

Key words: Osteoporosis, genetics, QTL, BMD, fracture, type-2 diabetes, GK rat, reciprocal cross, mtDNA

Classification system and/or index termes (if any):

Supplementary bibliographical information:

Language
English

ISSN and key title:
1652-8220

ISBN
978-91-86443-60-3

Recipient's notes

Number of pages
154

Price

Security classification

Department of Clinical Sciences, Malmö
Clinical and Molecular Osteoporosis Unit

Genetics of Osteoporosis

Studies on Bone Size, Structure and Strength in the Rat

Sofia Lagerholm

ISSN: 1652-8220
ISBN: 978-91-86443-60-3
Lund University, Faculty of Medicine,
Doctoral Dissertation Series 2010:45
Printed in Sweden by Media-Tryck, Lund University, 2010

CONTENTS

LIST OF PAPERS

I. Lagerholm S, Li LS, Jiao H, Park HB, Ohlsson C, Åkesson K, and Luthman H:
Genetic regulation of bone traits is influenced by sex and reciprocal cross in F2 progeny from GK and F344 rats.
Journal of Bone Mineral Research 2009 24:1066-1073

II. Lagerholm S, Park HB, Luthman H, Nilsson M, McGuigan F, Swanberg M, and Åkesson K:
Genetic loci for bone size determined by three-dimensional CT in crosses with the diabetic GK rat.
Submitted

III. Lagerholm S, Park HB, Luthman H, Grynpas M, McGuigan F, Swanberg M, and Åkesson K:
Genetic loci affecting fracture susceptibility interact with reciprocal cross in GK and F344 rats.
Submitted

IV. Park HB, Abhyankar A, **Lagerholm S,** Ivaska, K, Salehi A, Åkesson K, and Luthman H:
Effect of high-fat diet in a rat model for gene-diet interactions in obesity, type-2 diabetes and osteoporosis.
Manuscript

ABBREVIATIONS

AIL	advanced intercross line
BMC	bone mineral content
BMD	bone mineral density
BMP-2	bone morphogenic protein-2
CSA	cross sectional area
chr	chromosome
cM	centiMorgan
COLIA1	collagen type 1α1
CT	computed tomography
DIP	deletion/insertion polymorphism
DXA	dual energy X-ray absorptiometry
EC	endosteal circumference
ESRI	estrogen receptor α
F344	Fischer
GK	Goto-Kakizaki
IP	moment of inertia
IPGTT	intraperitoneal glucose tolerance test
LOD	logarithm of odds ratio
LRP5	lipoprotein receptor related protein-5
LR	likelihood ratio
mtDNA	mitochondrial DNA
mRNA	messenger RNA
PB	point bending
PC	peripheral circumference
PCR	polymerase chain reaction
PTH	parathyroid hormone
pQCT	peripheral quantitative computed tomography
RP	moment of resistance
TGFβ-1	transforming growth factor beta-1
VDR	vitamin D receptor
VNTR	variable length of tandem repeat
QTL	quantitative trait loci

INTRODUCTION

Bone structure

Up to 90% of bone is composed of an extracellular matrix, comprising an organic and an inorganic component. The organic component consists primarily of collagen, and type I collagen predominates. Collagens are responsible for the strength of the bone and the molecules have a characteristic triple helical conformation which extracellularly forms fibrils [1]. The remaining part of the extracellular matrix, the inorganic component, is an important and major reservoir of minerals in the body. The mineral salts are primarily calcium and phosphate in the form of hydroxyapatite [2]. Morphologically, bone consists of two different types of mineralized tissue, cortical bone and trabecular bone. Although their cellular and matrix components are similar, their structure and function differs.

The external shell of the bone and the middle of long bones (*the diaphysis*) consist of cortical bone, and represents nearly 80% of the skeletal mass. Cortical bone is compact and has a high resistance to bending and torsion, and important for the function of long bones. Trabecular bone (cancellous or spongy bone) is located in the vertebral bodies, *the metaphysis* of the long bones, and the inner parts of the small bones. It is a rigid meshwork of thin, mineralized trabeculae, less dense than cortical bone. The trabecular bone provides a large surface and it is the most metabolically active part of the skeleton. Bone turnover is thus greater in trabecular bone compared to cortical bone [3]. Despite the structural, distributional and functional differences, trabecular and cortical bone are produced by the same cell types and have the same overall matrix composition.

Bone tissue is continously being remodeled by the coupled processes of osteclastic bone resorption and osteoblastic bone formation. Remodeling allows bone to adapt to changes in the distribution of mechanical forces in response to mechanical and

physiological stress and to repair the microdamage that accumulates in bone matrix. A dynamic balance between bone formation and bone resorption is essential for the maintenance of skeletal integrity. Changes in bone remodeling and an imbalance between bone degradation and formation may result in bone diseases, including osteoporosis [4] **(Fig.1)**.

Osteoporosis

Osteoporosis is a complex disease, influenced by multiple genes and environmental factors. It is characterized by low BMD and deterioration of bone microarchitecture leading to increased bone fragility and increased susceptibilty to fracture [5].

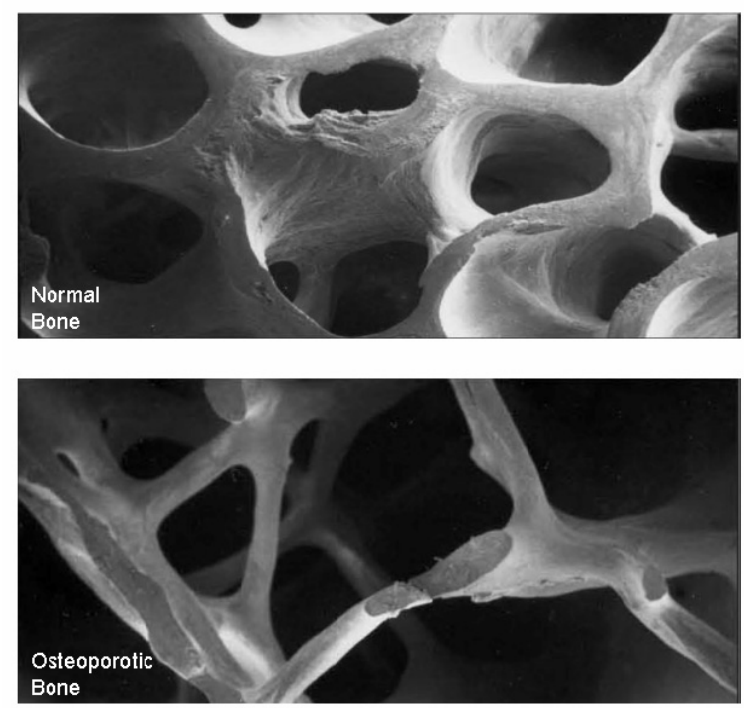

Fig.1. Normal trabecular bone and osteoporotic trabecular bone. (*Adapted and modified from Dempster DW, et al. J Bone Mineral Res 1:15-21.*

Osteoporosis is undoubtedly a major public health problem. High fracture rates result in the loss of quality of life in patients, but also place an economic burden on society. In Sweden, almost one half of all women and one quarter of all men will sustain a fracture after the age of 50 [6]. Therefore, it is imperative to develop inexpensive and widely applicable methods for diagnosis, prevention, and treatment to limit the increase in osteoporotic fractures.

Factors that influence fracture susceptibility

Several components affect bone strength and a bone's ability to resist fracture **(Fig.2)**, including the amount of material in the bone (i.e. size, mass, and density), its distribution (i.e. geometry and architecture), the intrinsic properties (i.e. composition) and its turnover (rate and balance of formation and resorption).

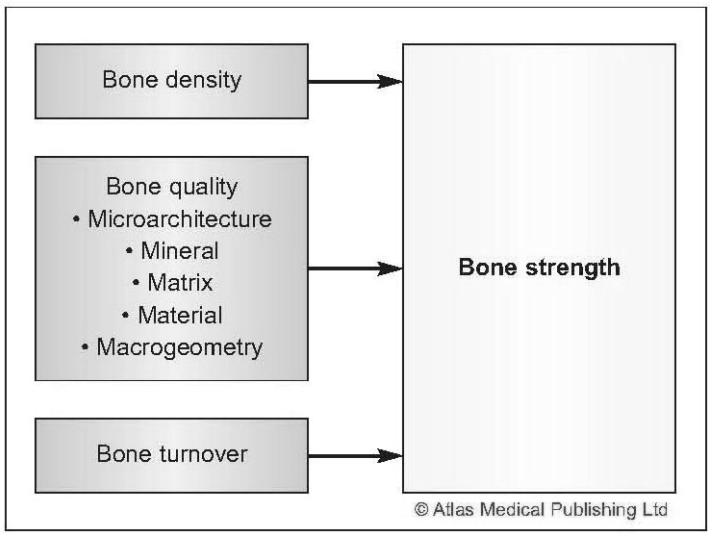

Fig.2. Factors that influence bone strength. *In courtesy of Clinical Publishing [7].*

Peak bone mass and bone loss

The amount of bone in all individuals increases during childhood and adolescence, during this period much more bone is deposited than withdrawn, subsequently the

skeleton grows in size and density. The amount of bone tissue in the skeleton continues to increase until around the age of 30 in men and women, when bone is considered to have reached its maximum strength and density, i.e. peak bone mass. Thereafter, in women, total bone mass tends to decrease between age 30 and menopause. Later during the first few years after menopause, women may also experience rapid bone loss, which then slows but continues throughout the postmenopausal years and through advancing age [8].

Risk factors

Apart from age and gender, the strongest risk factor for fracture is low bone mass. Body weight, lifestyle factors (nutrition, physical activity, smoking), medication, and genetic factors also contribute to the risk of osteoporotic fractures [9]. Additionally, diabetes has been described to impact bone through several mechanisms, with contradictory effects. Type-1 diabetes has been associated with decreased BMD as a result of insulin deficiency [10-11], while type-2 diabetes has been reported to have anabolic effects on bone resulting in higher BMD, due to hyperinsulinemia [12-13].

Osteoporotic fractures

Osteoporosis manifests clinically as fragility fractures that result from mild trauma acting on a skeleton with reduced bone strength. The most common sites for osteoporotic fractures are the hip, spine and distal radius. Loss of cortical bone predisposes an individual to fractures at the hip and wrist whereas the rate of trabecular bone loss is particularly associated with osteoporotic vertebral fractures [14]. The most severe fracture-type associated with the highest morbidity is hip fractures that occur mainly in elderly women [15].

Diagnosis

The definition of osteoporosis has since 1994 been based on recommendations from the World Health Organization (WHO) [5]. The diagnostic criteria for

osteoporosis are based on standard deviations (SDs) in BMD known as T-scores and indicate the difference between ideal peak bone mass achieved by a young adult and the bone mass of the patient. Individuals with BMD values lower than 2.5 SDs (T-score -2.5) from the mean of young adults are diagnosed with osteoporosis [16]. Additionally, a Z-score is used, which is based on a age and sex matched reference and used in children and adolescents before reaching peak-bone mass and sometimes in the very elderly. Several non-invasive methods are available for the assessment of the skeleton for the diagnosis of osteoporosis and the evaluation of fracture-risk. *Dual energy X-ray absorptiometry (DXA)* is the clinically established method used for measuring BMD. In DXA scanning, energy from X-ray beams is passed through the bone being examined. The denser the bone, the more energy is absorbed. The energy that is not absorbed is detected and determines the density of the bone. DXA measures the bone mineral content (BMC, g) and the areal bone mineral density (aBMD, g/cm^2). The density of the bone is determined by the mineral content, that is dependent on the amount of calcium phosphate at the specific site. DXA uses two different X-ray energies, that enable the measurement of density of skeletal tissue separately from the surrounding soft tissue [17]. However, the method gives information only in two dimensions and does not inform about the cortical and trabecular components. *Quantitative computed tomography (QCT)* provides the true measure of three-dimensional volumetric bone density (vBMD, g/cm^3) and allows the two main types of bone, trabecular and cortical bone, to be distinguished [18]. It is commonly used for the measurement of vertebral bodies. Other devices for the scanning of peripheral skeleton have also been developed including *peripheral QCT (pQCT)* that performs scans of the forearm and tibia.

Evaluation of fracture risk and intervention

It is essential that individuals with the highest fracture risk are identified. The ideal would be to identify and treat before the first fracture occurs, but for many the first

suspicion of osteoporosis is raised by a long-bone fracture following a fall, or a spontaneous vertebral fracture. People sustaining traumatic or low trauma fractures need to be assessed for the underlying cause to decide upon treatment to prevent further fracture. Age and female gender are the strongest risk factors for fracture. In addition, prior low energy fracture, use of certain medications, reduced lifetime oestrogen exposure, anorexia nervosa, low body mass index, maternal history of hip fracture, smoking, low physical activity and fall-related factors, such as visual impairment increase the risk of osteoporosis and fracture [19]. As the risk of fracture is increased in individuals with clinical risk factors and low bone density, the clinical risk factors can be used to identify who should be assessed for their future risk of fracture by bone densitometry and by new algorithms that are being developed [20].

Pharmacological treatment of osteoporosis

Pharmacological treatment of osteoporosis aims to reduce the number of fractures by improving bone mass. In general they either decrease bone resorption to produce secondary gains in bone mass or are anabolic and produce direct increases in bone mass. Antiresorptive treatment inhibits osteoclast function and reduces bone resorption. Since osteoblast function is unaffected, bone density increases. Anabolic treatments stimulate osteoblast function directly, while osteoclast function is unaffected, thereby increasing bone density. Antiresorptive treatments that reduce fracture risk include vitamin D, calcium, bisphosphonates, hormone replacement therapy (HRT; estrogen and estrogen combinations) and SERMs (selective estrogen receptor modulators). Bisphosphonates are the most extensively studied pharmacological treatment for osteoporosis, producing a fracture risk reduction of 30-50% [21], and are generally recommended as the first line of treatment. Parathyroid hormone (PTH) has bone anabolic properties when given intermittently and produces incremental increases in BMD but due to administration and costs, it is a second or third line treatment.

All fracture intervention trials show that those with the most pronounced osteoporotic disease and the highest fracture risk benefit most from treatment.

Genetics of complex diseases

Complex diseases are defined as being multifactorial, having both environmental and genetic risk factors. To identify the genetic components of a complex disease, co-inheritance of chromosomal regions associated with the disease is studied in families or at a population level. The human genome consists of approximately 22,000 protein coding genes. Genetic variation is determined by polymorphisms or mutations. To be classified as a polymorphism, the minor allele must have a frequency of 1% or more in the population. If the frequency is lower, the allele is regarded as a mutation. Approximately 90% of sequence variation among individuals is due to polymorphisms where the most common type are single-nucleotide polymorphisms (SNP). The remaining 10% of the genetic variants are deletion/insertion polymorphisms (DIP) and variable number tandem repeats (VNTR), known as microsatellites [22]. Since both SNPs and microsatellites are dispersed throughout the genome they can be used as genetic markers.

Coding DNA accounts for only about 1.5% of the human genome and most SNPs are therefore found in non-coding regions, such as within introns and may affect the regulation of gene expression [23]. However, SNPs in the coding regions of a gene can either change the amino-acid sequence or be synonymous or silent, which means that the codon change caused by the SNP does not result in an altered amino acid [24-25].

Genetic approaches

Two main approaches are used to identify genome regions underlying a phenotype associated with a specific disease: association studies in case-control materials and quantitative trait locus (QTL) mapping by genome-wide linkage analysis. In both of these, genetic markers with known genomic location are used.

Association studies in case-control materials test whether the frequency of alleles of a genetic marker differ between the two groups of individuals (subjects with disease and healthy controls) and can test for association between marker alleles and continuous phenotypes within a population. Association studies can be population or family-based and either require prior hypotheses about which genes to test or cover the entire genome (genome-wide association studies).

To perform *QTL mapping*, genetic markers must be identified and genotyped. Prior knowledge about the function of specific genes is, however, not required (random gene search). Quantitative traits are phenotypes that show continuous variation (e.g. BMD). QTL mapping aims to find markers that are statistically associated with the phenotype and linked to a chromosomal region that may contain one or more loci controlling the trait of interest [26].

The statistical significance of the QTL is calculated by LOD scores at many selected points in an interval between markers and plotted versus map position. The peak of the LOD score gives the most likely location of the QTL, and the height of the peak is a measure of statistical significance. The LOD score is defined as the log of the ratio of the likelihood of there being a QTL present vs. the likelihood of no QTL being present at a particular map position [27]. A LOD score higher than 3 (comparable to genome-wide $P<0.05$) is generally accepted as evidence for suggestive linkage in intercrosses between inbred animals [28]. Since many statistical tests are conducted in genome scans (multiple testing issues), the actual threshold for significance must be established by either permutation [29] or by numerical methods [28].

When the QTL is identified, candidate genes within the QTL can be identified that are functionally connected to the phenotype of interest. If the phenotype is determined by several QTLs, many genes of small effect influence disease susceptibility. Conversely, one single QTL can contain several genes affecting the same phenotype.

Gene mapping in animal models

The use of animal models as a complement to studies in human populations is one approach to accelerate the identification and functional characterisation of candidate genes. Animal models offer many advantages, including controlled breeding, standardized environmental conditions, the ability to study phenotypes similar to those observed clinically, and the ability to narrow the regions of QTLs by fine mapping by employing congenic breeding strategies [30-31]. Additionally, because the homologous regions of several animal genomes and human chromosomes are very well defined, it is possible to identify the chromosomal location of a candidate gene accurately in humans by mapping it in animal models **(Fig.3)**. The approach to identify a gene regulating a specific phenotype in an animal model is to localize QTLs for the phenotype, first in an F2 intercross, then in congenic strains carrying individual QTLs to study the underlying physiology of the phenotype, and in advanced intercross lines (AIL) for higher resolution in the positioning of the QTL. Once the region is narrowed, expression analysis combined with sequencing can be used to identify a potential candidate gene in the rat. Next step is comparative genomics i.e., to identify the syntenic human gene followed by functional analysis and finally test the gene in humans by association studies [31-32].

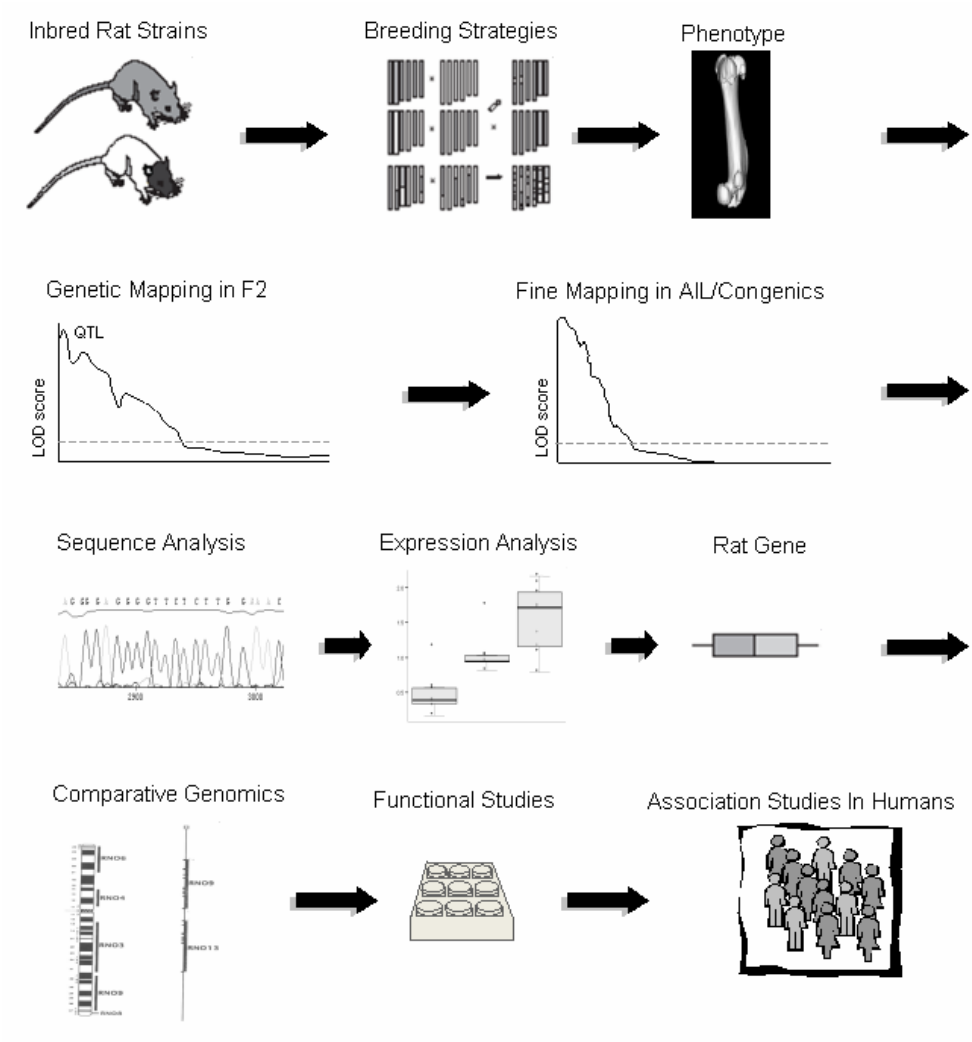

Fig.3. Pathway for the identification of genes for complex diseases in animal models. *Modified from Piehl et al, Physiol Behav. 2007 Sep 10;92:67-74.*

Breeding of F2, AIL and congenic strains

The *F2 progeny* is generated by crossing two strains, usually phenotypically distinct, to obtain a heterozygous F1 population. All rats in the F1 population are genetically identical, as they have inherited the same haplotype from each parental strain. For a reciprocal cross, the two groups of F1 progeny (A female x B male)

or (B female x A male) are then intercrossed separately to yield two reciprocal F2 populations **(Fig.4)**. From a genetic standpoint, the reciprocal crosses both harbour a mix of the two genomes (A and B) but differ in their mitochondrial genotypes as inherited from the founding female. Progeny displaying a quantitative trait associated with disease will give information as to which part of the genome is influencing the phenotype. Once a QTL has been identified it is important to confirm its location and eventually dissect the locus into such a small region that it will be possible to positionally clone the gene. This can be done using congenic strains. *Congenic strains* carry only homozygous genomic intervals, and are generated by transferring a specific genetic locus from a donor strain to a recipient inbred strain. Procedurally, the donor and recipient inbred strains are mated; the F1 progeny are then backcrossed to the recipient successively over ten generations (N10) with selection on the congenic fragment in each generation. With each generation, the homozygosity of the recipient background increases from 50% at N1 to 99.9% at N10 **(Fig.4)**. Another (or a complementary) strategy to narrow the relatively large QTLs identified in F2 whole genome scans, is the use of an *advanced intercross line (AIL)*. An AIL is created by repeated random intercrossing of at least 50 breeding pairs starting from the F2 generation from two inbred strains. Due to the higher density of recombinants, genetic mapping of an AIL requires dense genotyped markers. Although the breeding of an appropriate AIL requires many years, it is a valuable tool to fine-map QTLs with high-resolution **(Fig.4)**.

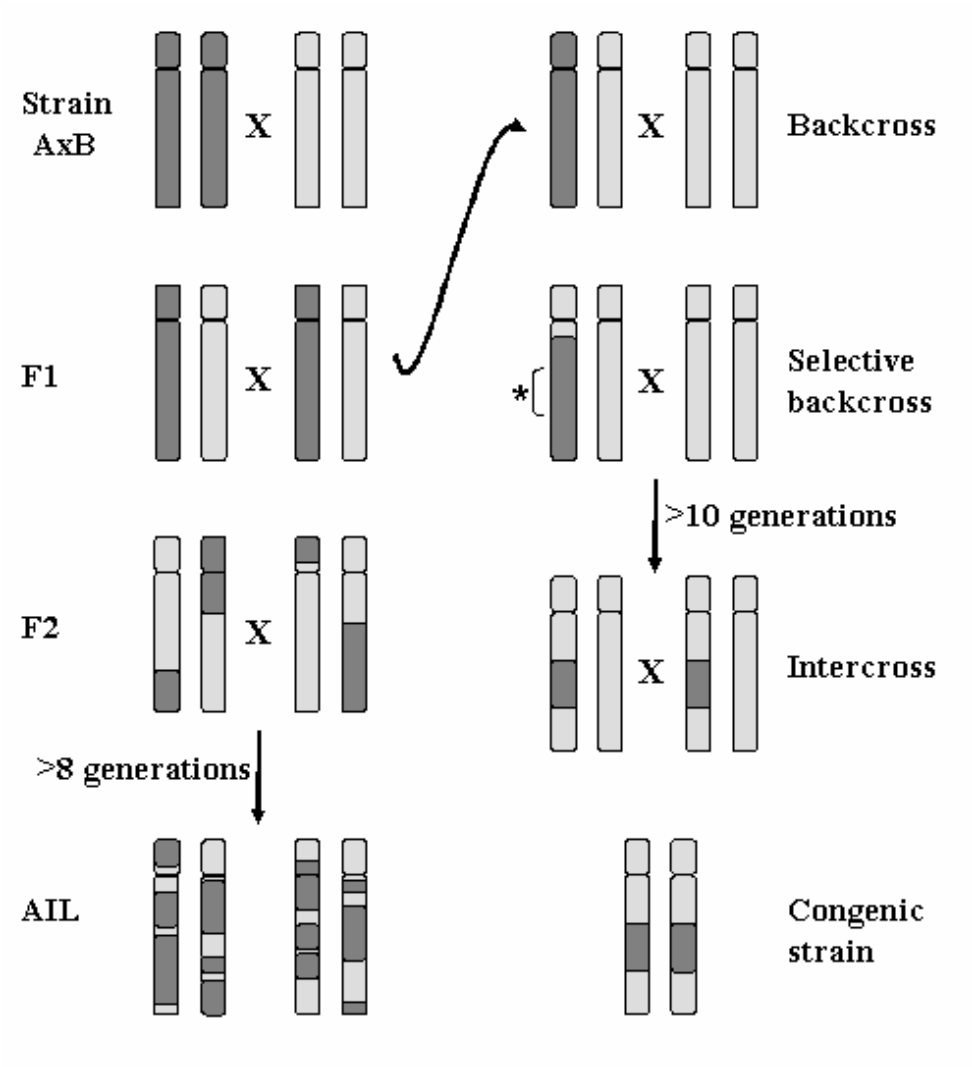

Fig.4. Breeding schedule of F2, AIL and congenic strains.

The genetics of osteoporosis

Bone strength is influenced by bone mass and bone quality, which encompasses both structural and biomechanical aspects. Family and twin studies have shown a significant heritable component, accountable for up to 80% with both BMD [33-35] and femoral structure [36], between 51% and 79% for hip axis length [37] and

from 60%-80% for the other aspects of bone geometry as well as bone quality [38] Data modelled in twins indicates that both specific and shared genetic factors act on individual bone phenotypes [39] and may explain the partially BMD-independent associations often observed with fracture. Several genome-wide linkage scans in humans have been performed in attempts to identify loci that regulate BMD [40-44]. Few of the genome-wide scans have identified QTLs that meet the criteria for genome-wide significance and there has been limited replication of QTL between different studies. Only one gene that regulates susceptibility to osteoporosis has been identified by this approach: the BMP-2 gene [43]. Nevertheless, some important findings have emerged from QTL studies, including evidence for a sex- and site-specific genetic regulation of BMD [41-42, 45-46]. The predominant strategy for identifying candidate genes for osteoporosis in humans is association studies. Although hundreds of association studies based on candidate gene polymorphisms have been performed, only a handful of genes with consistent effect on BMD, bone loss or osteoporotic fractures have been identified. These findings reflect the genetic heterogeneity and possibly the environmental variations in human populations, and frequently underpowered analyses which result in little consistency between studies [47]. Table 1 gives a brief overview of a few of the candidate genes related to osteoporosis phenotypes in association studies.

Table 1. Overview of candidate genes related to osteoporosis phenotypes.

Category	Candidate Gene	Reference
Calciotropic hormones and receptors	VDR (Vitamin D receptor) PTH (Paratyroid hormone) CTR (Calcitonin receptor) CaSR(Calcium secsing receptor)	[48-52]
Sex hormones and receptors	ESRα (Estrogen receptor α) AR (Androgen receptor)	[53-54]
Bone matrix components	COLIA1 (Collagen type 1α1) BGP (Osteocalcin)	[55-58]
Cytokines and growth factors	IGF-1 (Insulin like growth factor) IL-6 (Interleukin-6)	[59-60]
Wnt signaling pathway	BMP2 (Bone morphogenetic protein 2) TGFβ-1 (Transforming growth factor β-1) LRP 5 (Lipoprotein receptor related protein-5) SOST (Sclerostin)	[61-64]
Inflammation	ALOX 12 (Arachidonate 12-lipoxygenase) RANK (Receptor activator of nuclear factor-κB RANKL (RANK ligand) OPG (Osteoprotegerin)	[65-66]

Mice and rats to identify osteoporosis susceptibility genes

Inbred mouse and rat strains have been established as valuable models for dissecting the genetic regulation of bone. The variety of measurement tools available for measuring the many components of bone in mice and rats (i.e., peripheral- and full body DXA, pQCT, microCT, and biomechanical testing) have helped to identify strains with variations in bone strength, structure and BMD for genetic mapping, and several QTLs regulating these phenotypes have been

identified [67-77]. Additionally, genetic modifications of rodents are a powerful tool for studying genetics, with the possibility to arrange knock-in and knock-out models to test and confirm a specific gene function. One important finding from linkage analysis and knock-out models in mice, was the identification of ALOX15 (arachidonate lipoxygenase 15) as a candidate gene for the regulation of BMD [78]. Furthermore, studies in mice have shown that genes regulating BMD are site- and sex specific [79-80].

Observed variations in bone strength, structure and BMD among several inbred rat strains, together with progress in mapping the rat genome makes the rat a useful genetic model for skeletal fragility [81]. Compared to mice, rats are ideal for biomechanical analyses of bone strength due to larger bones thereby allowing more precise measurements.

The GK and F344 rat strains

The inbred GK (Goto-Kakizaki) rat is a well-established genetic model for type-2 diabetes. GK rats exhibit several features resembling diabetes in humans, such as fasting hyperglycemia [82] and impaired insulin secretion and action [83-85]. Progeny from F2-intercrosses arranged between GK and normoglycemic strains have been used for genome-wide linkage analyses, and significant QTLs for glucose tolerance have been identified on chromosomes 1, 2, and 10 [86-87]. Furthermore, bone changes in the GK strain have been observed, i.e. increased bone strength and loss of trabecular vBMD, making these rats well suited for investigations of possible interactions between bone and type-2 diabetes phenotypes. [88-90].

The normoglycemic Fischer (F344) rat strain has been shown to develop osteopenia similar to humans and to carry alleles contributing to skeletal fragility. F344 rats are therefore useful for studying genetic influences on bone strength and structure [77, 81, 91-93].

Genetic contributions from the mitochondrial genome

Mitochondria are the only organelles with their own DNA and this DNA is maternally inherited [94-95]. The mtDNA encodes 37 genes, including 13 protein-coding genes. These in conjunction with subunits encoded by the nuclear genome, form the electron transport chain, the primary adenosine triphosphate (ATP) producer in the cell. Consequently, mitochondria demand coordinated expression of both nuclear and mitochondrial genes to sustain full mitochondrial function. Therefore, mitochondrial dysfunction caused by impairment of the interaction between the two genomes is expected to affect the pathogenesis of metabolic disorders such as type-2 diabetes. However, studying nuclear-mitochondrial interactions in complex diseases like type-2 diabetes and osteoporosis in human populations is very difficult not only due to the large number of mildly deviant mtDNA haplotypes and the heterogeneous nuclear genome, but also the inability to control environmental factors that heavily influence these diseases. Inbred animals on the other hand offer a more standardized alternative to study such interactions. Recent studies identified mitochondrial influence on type-2 diabetes associated phenotypes by the use of conplastic rat strains, differing only in their mitochondrial genomes [96]. Another approach to study mitochondrial interactions is reciprocal crossing of two inbred strains [86].

In the mitochondrial genome of the GK and F344 rats, more than 100 variant positions segregating the strains have been identified. These include twelve non-synonymous amino acid changes in proteins required for ATP synthesis [97]. Recently, a reciprocal F2 cross between these two rat strains (resulting in two individual crosses with divergent maternally inherited mtDNA) was used for studying nuclear-mitochondrial interactions on type-2 diabetes related phenotypes (HB Park et al, unpublished). In this thesis, the same type of reciprocal cross between the GK and F344 rat is used to study the effects of reciprocal cross, i.e. mitochondrial genotype on the genetic regulation of bone phenotypes.

AIMS

The main aim of this thesis was to identify chromosome regions for several determinants affecting bone strength and fracture susceptibility, and to evaluate the effects of sex- and reciprocal cross on the genetic regulation of osteoporosis-related phenotypes. This was done in an F2 intercross between inbred diabetic GK and non-diabetic F344 rats. In addition, an AIL population of GK and F344 rats was tested as a model for the effect of a high-fat diet on bone.

Specific aims:

I. Identify QTLs affecting trabecular and cortical bone properties measured by pQCT in an F2 intercross of GK and F344 rats.

II. Characterize bone size phenotypes from parental (GK and F344), F1- and F2 rats using a new 3D CT method, and to identify QTLs contributing to the measured bone size parameters.

III. Identify QTLs linked to bone strength related phenotypes obtained by biomechanical testing and 2D DXA in an F2-intercross of GK and F344 rats.

IV. Evaluate the effects of a high-fat diet on diabetes and osteoporosis related phenotypes in AILs between GK and F344 rats.

METHODOLOGY

Animals

F2, F1 and parental strains of GK and F344 rats

An F2 intercross from inbred type-2 diabetic GK and normoglycemic F344 rats (108 males and 98 females) was studied in *Paper I-III*. Two separate F2 intercrosses were generated: one originating from grandmaternal GK and grandpaternal F344 (cross 1, (GK female × F344 male) F1), and the other from grandmaternal F344 and grandpaternal GK (cross 2, (F344 female × GK male) F1). The two groups of reciprocal F1 progeny were mated separately to yield two reciprocal F2-populations **(Fig.5)**. From a genetic standpoint, the reciprocal crosses differ by their mitochondrial genotypes as inherited from the founding female. All cross 1 progeny carry GK mitochondrial genotype, and F2-males carry Y-chromosome from F344, whereas females can only be heterozygous or homozygous for GK-alleles located on chr X. All cross 2 progeny carry F344 mitochondrial genotype, and F2-males carry Y-chromosome from GK, whereas females can only be heterozygous or homozygous for F344-alleles on chr X.

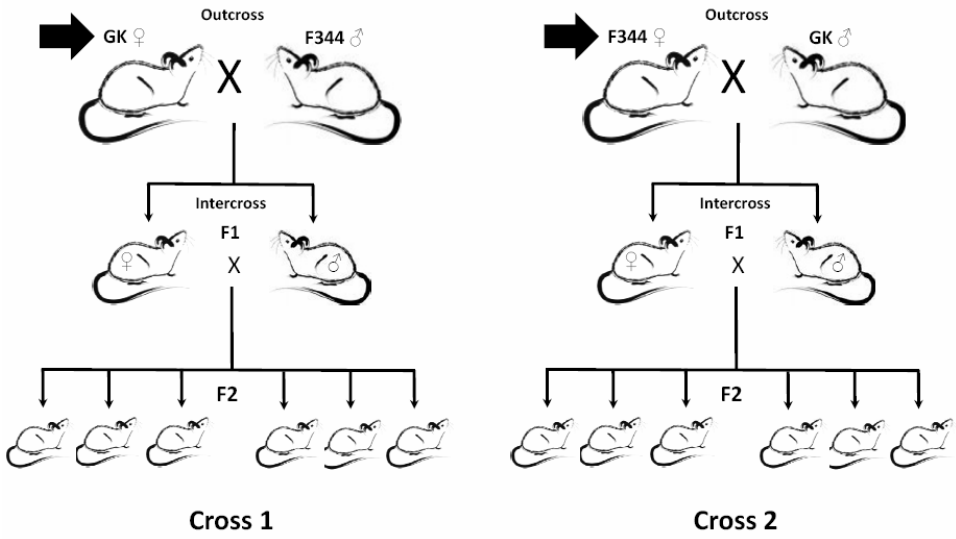

Fig.5. Breeding schedule for the F2 reciprocal intercross populations. *In courtesy of Avinash Abhyankar.*

In *Paper II*, males and females from the parental strains GK and F344 (n = 39) and F1 progeny from both reciprocal crosses (n = 37) were also included.

During the first four months all rats were fed normal protein rich chow, followed by a high-fat diet (supplemented with 2% cholesterol, 20% olive oil, and 0.5% bile acid, Lactamin AB, Linköping, Sweden), for three months. At a mean age of 215 days, the rats were sacrificed. For skeletal phenotypes, left femur and tibia from the parental- and F1 progeny and left tibia from the F2 progeny were collected.

AILs of GK and F344 rats

In *Paper IV*, a total of 70 male AIL rats were studied with the GK and F344 rats as parental strains. The F1 generation was generated with GK as female founders. The F2 progeny were generated from eight pairs of F1 rats. Continuous inter-crossing was conducted using randomly selected 50 breeding pairs to avoid brother-sister mating. 40 AIL at G20 generation and 30 at the G21 generation were used for the dietary intervention, with 60 rats introduced to a high fat diet (HFD) for 12 weeks while a

normal low-fat diet (LFD) were given to ten control rats during the same period. The experimental design for all rats is summarized in (**Fig. 6**).

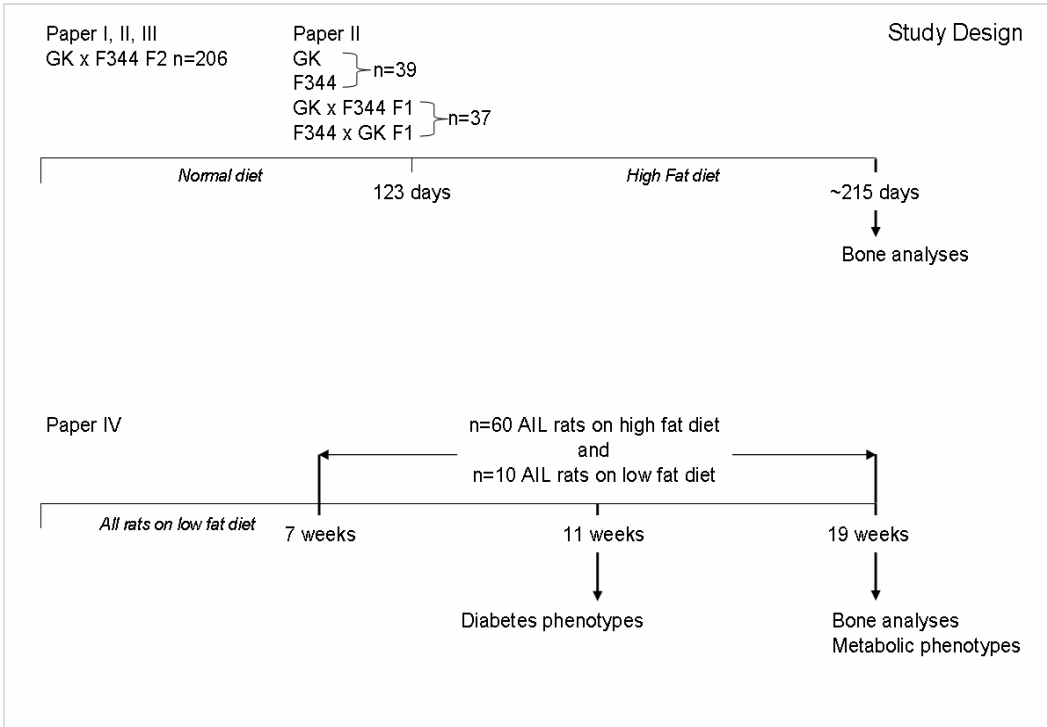

Fig. 6. Experimental design for Paper I-IV.

Metabolic and diabetes-related phenotypes

To evaluate the effects of the high-fat diet on metabolic phenotypes, plasma concentrations of fasting glucagon, total cholesterol and triglycerides were determined. For the effects on diabetes, measurements of fasting blood glucose, glucose tolerance and fasting insulin was longitudinally monitored along the dietary intervention.

In order to detect possible effects on bone metabolism of the high-fat diet, fasting plasma levels of osteocalcin was measured in the rats at the endpoint of the dietary

intervention study. Osteocalcin is a bone-specific protein produced by osteoblasts during bone formation and can be used in clinical investigations as a marker of bone metabolism. Elevated osteocalcin is associated with both high bone formation and high bone turnover [98]. Furthermore, recent evidence in mice suggests that bone participates in the regulation of glucose homeostasis and insulin sensitivity via osteocalcin [99].

Bone Analyses

Dual Energy X-ray absorptiometry (DXA)

DXA is the key diagnostic tool in osteoporosis and measures bone mineral density (BMD). The method measures the amount of radiation absorbed by a particular anatomical surface. The absorption is proportional to the bone mineral of the area exposed to the radiation, and the content of the area provides the bone density value expressed in g/cm^2. The method is based on projected radiographic images in two dimensions and does not inform about bone material quality and architectural structure such as cortical and trabecular components [17].

Tibias from the F2 progeny in *Paper III*, were scanned by DXA using the PIXImus^{TM} densitometer (GE Lunar Madison, WI) specially modified for use on small specimens and generated phenotypes including areal BMD (g/cm^2), BMC (g) and projected area (cm^2). Additionally, whole-body DXA (Lunar DPX-L, Lunar Corp., Madison, WI), generating the following parameters: whole-body BMC (g), areal BMD (g/cm^2), area (cm^2), total body fat (%), tissue (g) and lean mass (g), was performed for the parental- and F1 progeny of GK and F344 rats **(Table 1A and 1B in Appendix A**) and for the AILs in *Paper IV*.

Peripheral Quantitative Computed Tomograhy (pQCT)

In contrast to DXA, pQCT offers 3-dimensional information, and trabecular and cortical compartments can be separately measured in defined regions of a bone. Software specially modified for use on small bone specimens makes it possible to scan samples from mice and rats.

In *Paper I*, tibia from the F2 rats were measured by pQCT using a Stratec XCT Research M (Norland, Fort Atkinsson, WI, USA) with a voxel resolution of 70 microns modified for use on small bone specimens (Software 5.40B). The length of tibia was measured using a caliper. Two pQCT scans were performed, one proximal metaphyseal and one mid-diaphyseal **(Fig.7)**. For trabecular bone

analysis, one scan at a distance equal to 5% of the length in the distal direction from the proximal tibia growth plate was performed (proximal metaphysis). The trabecular bone region was defined as the inner 45% of this area and generated trabecular vBMD (g/cm^3), total metaphyseal vBMD (g/cm^3), and cross sectional area (CSA, mm^2) of the whole proximal metaphyseal area. Cortical diaphyseal bone was measured at a position 40% of the total bone length in the distal direction from the proximal tibia growth plate and provided the following phenotypes: cortical vBMD (g/cm^3), cortical thickness (mm), cortical BMC (mg/mm), cortical CSA (mm^2), periosteal circumference (PC, mm), and endosteal circumference (EC, mm). Biomechanical strength was calculated from the cortical diaphyseal data: cross-sectional moment of inertia (IP, mm^4) and cross-sectional moment of resistance (RP, mm^3).

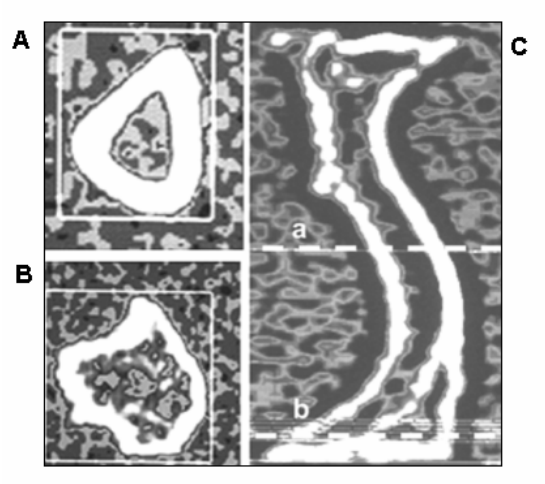

Fig.7. pQCT scan of mouse tibia.

(A) Cross-sectional image at mid-diaphysis.

(B) Cross-sectional image at proximal metaphysis.

(C) Scout view showing the position of the two scans.

Adapted from Schmidt C et al. 2003 J Bone Miner Res 18: 1486-1496.

Three-dimensional Computerized Tomography (3D CT)

In *Paper II*, we used a new 3-dimensional CT (3D CT) method to measure bone size phenotypes. Compared to the previously described methods (DXA and pQCT), the CT method provides additional information about bone size characteristics of both the cortical and the entire bone. Tibia from males and females from the parental, F1 and F2 generations were included for bone size measurements using the 3D CT method. Additionally, femur from the parental- and F1 progeny of GK and F344 rats were also included for 3D CT **(Table 2A and 2B in Appendix A)**.

The bones were placed in 70% ethanol in 15 mm diameter plastic tubes and scanned in a CT unit (Somatom Sensation 64, Siemens Ag, Erlangen, Germany). Images were acquired using 120 kV and 140 mAs per revolution with collimation 12 · 0.6 mm and pitch 0.8. The field of view was reduced to 50 mm for maximum geometric resolution. Images with a slice width of 2 mm were reconstructed using reconstruction kernel "u80u" with a reconstruction increment of 0.4 mm. The images produced with the CT scanner therefore represent a 3D voxel matrix with a resolution of 0.1 · 0.1 · 0.4 mm.

The images were processed using the Analyze (version 5.0) software package (Biomedical Imaging Resource, Rochester, MN, USA). The 3D voxel matrix was resampled using interpolation to an isotropic resolution of 0.1 · 0.1 · 0.1 mm. The bone samples were extracted from the 3D matrix with a volume rendering procedure using Hounsfield value thresholds for extracting either all (cortical and trabecular) bone tissue or only the cortical regions.

The following measurements were made on the bones: Total and cortical bone volume (mm^3), cortical bone volume fraction (%), total and cortical mean Hounsfield number, straight and curved length (mm) and peri- and endosteal area at mid-shaft (mm^2). Peri- and endosteal area at fibula-site (mm^2) was also measured, primarily because in these small bones it was a distinct site and likely to

correspond to the transition between metaphyseal and diaphyseal bone, a common fracture site in humans **(Fig. 8)**.

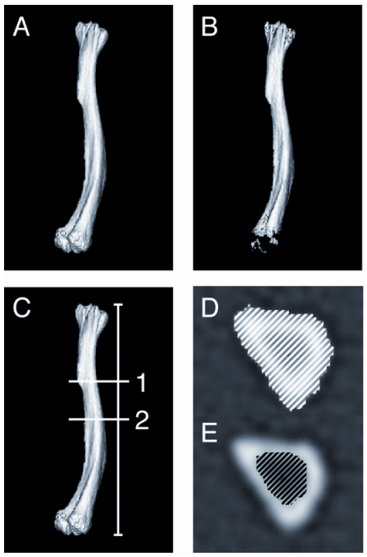

Fig. 8. Definition of measured CT variables: (A) Total bone volume (mm^3) and vBMC (mg/cm^3). (B) Cortical bone volume (mm^3) and vBMC (mg/cm^3). (C) Total straight and curved length (mm). (D) Periosteal area (mm^2), i.e., the cross-sectional area delineated by the outer circumference and (E) endosteal area (mm^2), i.e., the cross-sectional area delineated by the inner circumference at (C1) fibula-site and (C2) mid-shaft of tibia.

The BMC (mg/cm^3) was calculated using a calibration method previously developed for determining BMC in human vertebrae [100]. Since the calibration of the CT unit is dependent on the size of the scanned object, the calibration equation was re-calculated to ensure validity for the very small objects used in this study. This was performed by scanning the BMC calibration standards in the same geometry as the bone samples, allowing total and cortical measured BMC (mg/cm^3) to be calculated from the respective mean Hounsfield value measurements.

Both reproducibility and accuracy were tested for the 3D CT method. The reproducibility of the analytical procedure was tested by repeating the analysis of the first scan using the same rats (n=40) to calculate intra-observer coefficient of variation (CV) for each analyzed parameter. A second scan at a different time point from the initial measurement was performed, repeating the measurements of the left tibia from 5 male and 5 female rats of each strain and cross (n=40). The

duplicate scans and the subsequent analysis were made by the same operator. Additionally, we evaluated if the method was equally reliable in high and low size intervals by analyzing ten rats of varying body size for periosteal mid-tibial circumference in order to calculate the correlation with previous mid-tibial areal measurement.

For the purpose of determining the accuracy of the CT method for volume determination, objects with known volumes were scanned, using the same scanning parameters as for the rat bones. The objects consisted of plexiglass rods with carefully measured dimensions. The diameter and length of the objects were measured with a digital sliding caliper (Mauser, Digital 6, 8M007906, Switzerland) which in its turn was calibrated against a calibration object (series 167-102, Mitutoyo Corp., Kawasaki, Japan) with a length of 50 ± 0.002 mm.
The plexiglass rods had volumes of 180.3, 320.5 and 494.8 mm^3 when measured with the caliper. The corresponding values when measured with the 3D CT method were 179.6, 319.7 and 494.5 mm^3, respectively.

Biomechanical testing

For biomechanical properties of tibia in the F2 rats, the three point bending test was used in *Paper III.* This method is based on testing how much mechanical load a bone can sustain before fracturing and provides information about breaking force, stiffness and breaking strength. These parameters appear to be good indicators of the mechanical strength of cortical bone [101].

Prior to testing, tibia were thawed and equilibrated at room temperature (~2 hours). Tibia were positioned on the lower supports of a three-point bending fixture approximately 16 mm apart and held in a stable position by a 2N preload. Using a material testing machine (Instron 4465, Norwood, MA) with a 1KN load cell, the bones were loaded at their midpoint and at a deformation rate of 1mm/min until fracture. Load-displacement data representing structural or extrinsic properties of the bone were calculated from load-displacement curves and collected using LABView [102]. Parameters included: ultimate force (N; height of curve) reflecting the maximum load the bone can absorb before failing (i.e. bone strength), stiffness (N/mm; initial slope of the load displacement curve), and work to failure (mJ; area under curve) reflecting the total energy the bone can absorb before fracture **(Fig.9)**.

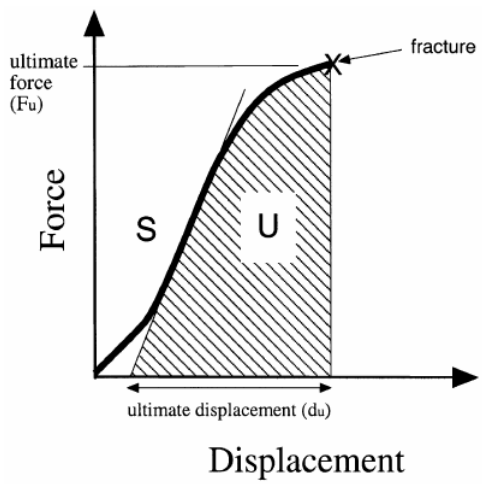

Fig. 9. A force displacement curve resulting from a biomechanical test of a specimen. The height of the curve (ultimate force) represents strength, area under curve is the work of failure (U), the maximum slope of the curve is stiffness (S).
*Adapted from Turner CH, 2002 Osteop Int.13:97-104*3.

Genetics and statistical analyses

DNA isolation and genotyping

Genomic DNA was purified from rat liver using QIAmp DNA mini kit (QIAGEN, Valencia, CA, USA). Genotyping of the F2-intercross (108 male and 98 female rats) was accomplished using PCR with microsatellite markers shown to be polymorphic in GK and F344 rats [103]. PCR was performed with one primer in each pair fluorescently labelled (hex/fam) (DNA technology, Aarhus, Denmark) and for flourescent detection, electrophoresis was performed on an ABI3000 Sequencer (Applied Biosystems). The ABI software GENESCAN was used to determine genotypes. A total of 192 genome-wide microsatellite markers at an average spacing of 9.3 cM were included and a genetic linkage map of the 20 rat autosomes was generated using MAPMAKER/EXP [104] and a map of the X chromosome using R/qtl [105]. The total genetic map length was 1784.2 cM (autosomes 1697.9 cM plus 86.3 cM for the X chromosome).

QTL identification

All phenotypes were normally distributed or log-transformed to obtain a normal distribution. To compare the bone phenotypes between males and females and between the reciprocal crosses, one-way ANOVA was used. The level of significance was set at $p<0.05$. Unless stated, *p*-values are nominal. Phenotypes were adjusted for reciprocal cross, age, litter size, and body-weight using regression analyses. Residuals were checked for normality and used in the QTL analysis. To account for gender attributable to bone quality differences, the residuals were computed separately for each sex.

The QTL analyses in *Paper I-III*, were performed for each sex separately. In order to identify possible interaction differences between loci in the nuclear genome and mitochondrial DNA, the sex separated F2 progeny was also separated on the basis of reciprocal cross. QTLs on autosomes were identified employing MAP MANAGER/QTX v. b20 [106]. The X chromosome was also included in our

linkage analysis using R/qtl [105]. Mapping QTLs on the X chromosome was conducted for each sex separately without further separation by cross. Permutation tests were performed to establish genome-wide significance levels by randomization of the phenotypic data in relation to genotypic data [29]. Significant (i.e., genome-wide false-positive rate of <5%) and suggestive (i.e., genome-wide false-positive rate of <63%) linkage was employed to establish genome-wide thresholds [107-108].

Identification of sex- and cross specific QTLs

To identify sex-specific QTLs, the LOD score differences between males and females across the genome were assessed (ΔLOD_{sex} score). A permutation method was applied to evaluate sex-specific QTLs, where thresholds were established using two randomly selected equal sized subsets of males and females [109]. The randomization was conducted within each cross. Subsequently, the bone phenotypes in the two subsets were permutated to calculate average ΔLOD_{sex} scores for genome-wide significant sex-specificity at suggestive (α=0.63) and significant (α=0.05) levels across the genome. Genetic markers on the X chromosome were not included in the permutation tests.

Within each sex, subsequent reciprocal cross-separated linkage analyses were conducted to identify cross-specific QTLs. The LOD score differences between cross 1 and cross 2 (ΔLOD_{cross} score) across the genome were evaluated. Thresholds of the cross specific QTLs were computed by permutation using two randomly selected equal sized cross 1 and cross 2 subsets.

To confirm the sex- and cross-specific QTLs identified with the ΔLOD method, likelihood ratio tests were performed comparing a full model with a QTL×sex interaction term / cross interaction term and a reduced model without the interaction term, using both male and female data for sex interaction and each sex separately for cross interaction.

$$Y = \beta_0 + CZ + \beta_1 X + \beta_2 QTL + \beta_3[QTL \times X] + e \quad \text{(Full model 1)}$$

$$Y = \beta_0 + CZ + \beta_1 X + \beta_2 QTL + e \quad \text{(Reduced model 1)}$$

Where, Y is the phenotype; β_0 is the mean; C is a vector of regression coefficients for cross / -, age, litter size, and body-weight; Z is a matrix of regression variables for cross / -, age, litter size and body-weight; β_1 is a regression coefficient for sex / cross; X is the regression variable for sex / cross; β_2 is a regression coefficient for QTL; QTL is regression variable for QTL; β_3 is a regression coefficient for the QTL-by-sex interaction / QTL-by-cross interaction; QTL×X is the regression variable for the QTL-by-sex interaction / QTL-by-cross interaction; and, e is the residual error.

Residuals of each phenotype were examined for normality with normal probability plots. The level of significance for a specific QTL interaction with sex or cross was set at $p<0.05$.

Statistical power calculations regarding the sample size were performed using the method of Lynch and Walsh [110]. We assumed that QTL acts additively in this power calculation. The fraction of phenotypic variance explained by QTL (i.e., R^2) was considered as effect size of QTL. Using a LOD score of 2.4 to control false positive detection of linkage, a sample size of 52 is necessary to achieve 80% statistical power for detecting a QTL with R^2 value of 0.25.

Table 2. Overview of the study design for Paper I-IV.

	Rats	**Phenotype (Bone / Diabetes)**	**Genotyping**	**Analysis Genetic / Biochemical**
Paper I	GK x F344 F2 (*N = 206*)	□ pQCT (tibia)	192 microsatellite markers	QTL analysis
Paper II	GK x F344 F2 (N = 206) GK x F344 F1 GK, F344 (*N = 76*)	□ 3D CT (tibia) □ 3D CT (tibia, femur) □ Whole-body DXA	192 microsatellite markers	QTL analysis
Paper III	GK x F344 F2 (*N = 206*)	□ Three-point bending □ 2D DXA (tibia)	192 microsatellite markers	QTL analysis
Paper IV	AILs of GK and F344 rats (*N = 70*)	□ Whole-body DXA □ Fasting glucose □ Insulin □ Glucagon □ Cholesterol □ Triglyceride		Bone turnover marker -Osteocalcin

RESULTS

Study I

Genetic regulation of bone traits is influenced by sex and reciprocal cross in F2 progeny from GK and F344 rats.

The aim was to identify genome regions linked to trabecular and cortical bone phenotypes in F2-intercross of inbred GK and F344 rats, and to investigate effects of sex and reciprocal cross.

A genome wide screen of QTLs associated with pQCT measures of tibia was completed in 108 male and 98 female F2 rats, comprising reciprocal crosses with divergent mtDNA, either from GK or F344.

Strong sexual dimorphism was observed for all pQCT traits in the F2 progeny with significantly higher values in males, except for total BMD which was 18% higher in females. Significant phenotypic differences were also observed between F2 progeny from the two reciprocal crosses, with predominately lower values in cross 2 progeny (F344 grandmaternal origin).

Four genome-wide significant QTLs linked to either cortical vBMD, tibia length, body length, or metaphyseal area were identified in males on chromosomes (chr) 1, 8, and 15. In females, three significant QTLs linked to cortical BMC, or metaphyseal total vBMD were identified on chr 1 and 2. A broad region on chr 1 (10-93 cM) showed linkage to several parameters of cortical bone size in both sexes (CSA, BMC, IP, RP, and PC) and overlap QTLs for fasting glucose.

Our analysis of sex- and reciprocal cross effects with likelihood ratio tests generated four female-specific QTLs on chr 2, 3, 5, and 10 **(Table 3)** and four reciprocal cross-specific QTLs on chr 1, 10, and 18 **(Table 4B)**.

The reciprocal cross specific QTLs were identified in females and were only expressed in cross 2, indicating that segregation of the QTLs can only be observed in the presence of F344 mtDNA.

In summary, these results provide evidence that both sex- and reciprocal cross, most probably the mitochondrial genotype influence the expression of cortical and trabecular bone. Of particular interest is the identified QTL region on chromosome 1 (10-93 cM) that affected several pQCT phenotypes and fasting glucose, suggesting a potential genetic association between bone- related phenotypes and traits affecting type-2 diabetes.

Study II

Genetic loci for bone size determined by three-dimensional CT in crosses with the diabetic GK rat.

The aim of this study was to test reproducibility and accuracy of a new 3D CT method measuring bone size parameters in rats and to identify quantitative trait loci (QTLs) regulating these phenotypes.

Tibia from male and females, representing the parental, F1 and F2 generations were characterized for bone size using the 3D CT method. The CT results between parental GK and F344 in both males and females, demonstrated overall elevated tibial bone size phenotypes in the GK rat. No pronounced difference was observed for the F1 progeny, with the exception of curved tibia length, that was significantly higher (+6.7%, p=0.009) in females having GK mtDNA. In the F2 progeny, strong sexual dimorphism was observed for all traits. All CT traits were significantly higher in males. Phenotypic differences were also observed between F2 progeny from the two reciprocal crosses, with more pronounced differences related to grandmaternal origin in female rats. Total and cortical volume, cortical bone volume fraction, straight length and cortical midshaft CSA were all higher in the cross carrying GK mtDNA (*p*-values 0.02-0.009).

In order to better estimate the genetic effects of the parental- and F1 progeny of GK and F344 rats, these strains were included for additional bone-characterization including femur measured by 3D CT and whole-body DXA. This information will be useful in the future breeding of congenics of the GK and F344 rat **(Table 1A, 1B, 2A, 2B in Appendix A)**. Genetic mapping of the bone size phenotypes in the F2 progeny generated several significant QTLs in both sexes on chr 1, 3, 4, 9, 10, 14, and 17. Suggestive QTLs are summarized in Table 3A and 3B **(Appendix A)**. Overlapping QTLs for both males and females in the (GK×F344) F2 progeny were located on chr 1 (26-97cM). This region overlapped previously reported pQCT

QTLs and overlaps loci for fasting glucose. Sex specific analysis confirmed a male suggestive QTL on chr 9 (67-82 cM) for endosteal area at the fibula site. Analyses separating the F2 population both by sex and reciprocal cross identified cross specific QTLs on chr 14 in males **(Table 4A)** and on chr 3 and 4 in females **(Table 3)**. Evaluation of the 3D CT method showed overall very low parameter dependent variability on repeated scans, with CV's ranging from 0-8.2%. Intra-observer analysis yielded variations between 0.4-9.2%. The cross-sectional endosteal areal measurements had the largest variation, nevertheless area and periosteal circumference were highly correlated (r=0.997).

In summary, we identified novel sex- and reciprocal cross specific QTLs for bone size phenotypes measured by a new application of 3D CT.

The results from the validation of the 3D CT method demonstrated that this method delivers highly reproducible bone size measurements with high precision in the rat. The most interesting regions were identified on chr 1 and chr 4. The region on chr 1 overlapped previously identified QTLs for pQCT phenotypes; and the reciprocal-cross specific QTL on chr 4 overlapped a previously identified locus linked to femoral phenotypes in rats, confirming these regions to contain genes of importance for bone regulation. The observed interaction between nuclear QTLs for bone size and reciprocal cross motivates further investigation of mitochondrial effects on bone.

Study III

Genetic loci for determinants affecting fracture susceptibility in crosses between GK and F344 rats interact with reciprocal cross.

The aim of this study was to identify QTLs for biomechanical strength by three-point bending (3PB) testing and 2D DXA phenotypes in the F2 progeny (previously used for identification of pQCT and CT QTLs), and to further delineate the effects of sex- and reciprocal cross on the genetic regulation of these phenotypes.

In male rats, LOD-scores of 4.7 and 4.1 were detected on chr 8 linked to tibial area and BMC. In females, the maximum LOD-score was 5.5 on chr 1 with linkage to stiffness and suggestive linkage to area, BMC and ultimate force. However, none of these QTLs reached significance for sex-specific interactions.

The reciprocal cross-separated QTL analyses in each sex generated additional significant QTLs, on chr 15 (ultimate force, LOD=3.9, males) and on chr 4 (BMC, LOD=5.1) in females with grandmaternal GK origin. These QTLs would not have been detected in the combined sample including both reciprocal crosses. Additionally two QTLs were identified in all males on chr 2 (aBMD) and chr 6 (BMC) at a suggestive level but showed significant linkage with LOD scores of 4.7 and 4.8 in the cross with F344 grandmaternal origin. Subsequent likelihood ratio tests confirmed cross-specific interactions for the QTLs identified on chr 2, 6 and 15 in males **(Table 4A)**.

By combining the identified QTLs in this study with previously identified QTLs linked to pQCT and 3D CT phenotypes, overlapping regions between all phenotypes were detected on chr 1, 3, 4, 6, 8, 10 and 14. The identified region on chromosome 1 (17-79 cM) displayed linkage to bone phenotypes obtained from all four methods (pQCT, CT, DXA and 3PB) and substantiate further support for this region being important for bone regulation **(Fig.10)**.

The co-localized QTLs on chr 4 and chr 6 were consistently detected in either sex and showed more significant linkage in one reciprocal cross depending on the mtDNA origin. In conclusion, the observed interactions with both sex- and reciprocal cross that could most likely be explained by mtDNA variation, demonstrate two important factors to be considered when interpreting the genetic regulation of phenotypes affecting fracture susceptibility.

Summary of results (Studies I-III)

Table 3. Summary of sex-specific QTLs.

Chr	QTL region[a] (Position, cM)	Method	Phenotype	LOD (LR; p-value[b]) Male	Female
1	D1Wox16-D1Mgh1 (66-93)	pQCT	MetavBMD		4.0 (12.1;0.002)
2	D10Rat23-D2Mgh22 (116-135)	pQCT	MetavBMD		4.1 (8.7;0.013)
3	D3Rat49-D3Mit8 (9-33)	pQCT	MetavBMD		3.6 (7.3;0.026)
5	D5Mgh21-D5Mgh16 (88-108)	pQCT	MetavBMD		3.1 (13.5;0.001)
9	D9Mgh2-D9Rat4 (67-82)	3D CT	EAfib	4.1 (19;10^{-4})	
10	D10Mit10-D10Rat27 (30-59)	pQCT	MetavBMD		3.2 (9.1;0.01)

[a]The approximate size of the QTL was defined as the region covered by a 1-LOD reduction for any of the bone traits

[b]Sex-specific QTLs (ΔLOD>2.4) , validated by likelihood ratio (LR) tests for QTL-by-sex interaction (p<0.05)

Table 4A. Summary of reciprocal cross-specific QTLs in males (Studies I-III).

Chr	QTL region[a] (Position, cM)	Method	Phenotype	LOD scores (LR, p-value[b]) Males Cross 1	Males Cross 2
14	D14Mit10-D14Rat22 (72-84)	3D CT	CAfib		3.9 (10.7;0.005)
15	D15Rat109-D15Mit12 (10-32)	3PB	Ult force	3.9 (10.4;0.005)	

[a]The approximate size of the QTL was defined as the region covered by a 1-LOD reduction for any of the bone traits

[b]Reciprocal cross specific QTLs (ΔLOD>2.4), validated by likelihood ratio (LR) tests for QTL-by-cross interaction (p<0.05)

Table 4B. Summary of reciprocal cross-specific QTLs in females (Studies I-III)

Chr	QTL region[a] (Position, cM)	Method	Phenotype	LOD scores (LR, p-value[b]) Females Cross 1	Females Cross 2
1	D1Rat4-D1Rat176 (10-27)	pQCT	MetavBMD		4.5 (7.0;0.03)
1	D1Mgh1-D1Wox20 (66-93)	pQCT	MetaCSA		3.3 (8.3;0.016)
2	D2Mit24-D2Mgh5 (45-57)	2D DXA	aBMD		4.7* (10.4;0.005)
3	D3Mit10-D3Rat46 (0-14)	3D CT	PAfib	4.8 (9.7; 0.008)	
4	D4Mit9-D4Mit24 (37-48)	3D CT	CortBV	4.9* (12.1; 0.002)	
4	D4Mit9-D4Mit24 (36-48)	3D CT	TotBV	5.6* (7.9; 0.02)	
6	(D6Mgh11-D3Mit19 (25-58)	2D DXA	BMC		4.8* (10.9;0.004)
10	D10Mgh13-D10Rat2 (88-100)	pQCT	CortPC		3.1 (7.5;0.023)
10	D10Mgh13-D10Rat2 (86-100)	pQCT	CortEC		3.3 (8.1; 0.017)
18	D18Mit2-D18Mgh5 (21-31)	pQCT	MetaCSA		3.0 (11.1;0.004)

[a]The approximate size of the QTL was defined as the region covered by a 1-LOD reduction for any of the bone traits.

[b]Reciprocal cross specific QTLs (ΔLOD>2.4); validated by likelihood ratio (LR) tests for QTL-by-cross interaction (p<0.05).

*Significant reciprocal cross specific QTLs (ΔLOD>3.9); validated by likelihood ratio (LR) tests for QTL-by-cross interaction (p<0.05).

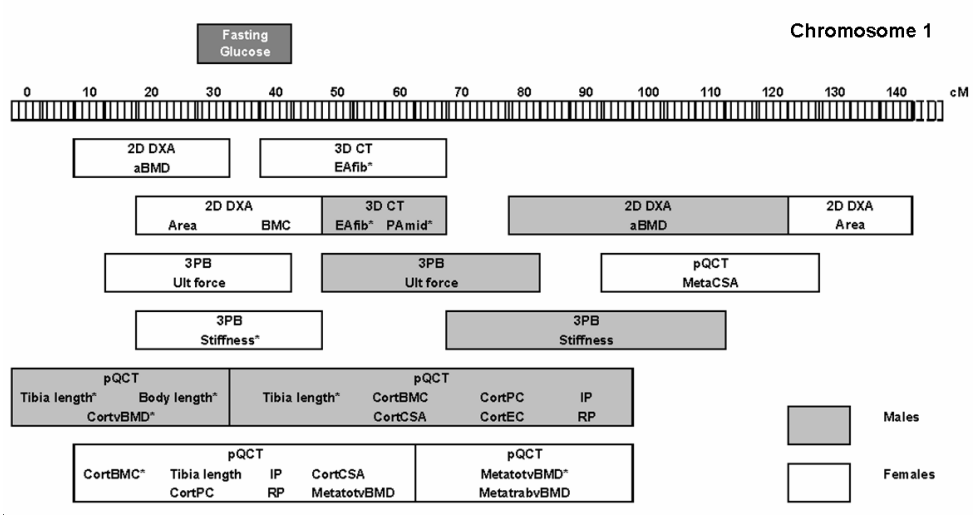

Fig. 10. Summary of all QTLs identified on Chr 1 linked to bone phenotypes measured by 4 different methods and location of the overlapping QTL linked to fasting glucose [111] identified in GK x F344 F2 rats.
*Indicate genome-wide significant QTLs (LOD>3.8).
All other presented QTLs reached genome-wide suggestive linkage (LOD>2.4).

Study IV

Effect of high-fat diet in a rat model for gene-diet interactions in obesity, type-2 diabetes and osteoporosis.

Since possible associations have been reported between type-2 diabetes and osteoporosis, it is relevant to identify the genetic factors of complex diseases in conjunction with environmental and lifestyle factors in order to develop more effective approaches to prevent and treat the diseases. A high-fat diet is one such powerful factor. Changes in dietary behavior have lead to widely recognized increases in obesity-related medical problems in western societies.

Therefore, the aim of this study, was to experimentally test the phenotypic responses of a high-fat diet on obesity-induced diabetes and osteoporosis in advanced intercross lines (AILs) between diabetic GK and non-diabetic F344 rats.

G20 and G21 generation AIL males were used for the dietary intervention, with 60 rats introduced to a high fat diet (HFD) for 12 weeks and compared to a normal low-fat diet (LFD) given to ten control rats during the same period.

The final outcome on bone from the dietary intervention was evaluated by whole body BMD measurements and plasma carboxylated osteocalcin as a marker of bone turnover. Additionally, the response to the HFD was longitudinally monitored; body weight, fasting glucose, insulin and other metabolic markers.

Compared to the LFD group, energy consumption, body-weight and adiposity in the HFD group were significantly higher after 12 weeks of HFD. Bone mineral density was lower in the HFD group, while no significant difference in osteocalcin levels was observed. Mixed-effects model analysis showed progressively elevated fasting insulin levels over time in response to HFD. The HFD rats showed higher levels of total cholesterol, while no effect on triglyceride and lower levels of fasting glucagon.

In summary, our findings demonstrate that increased energy intake due to the HFD treatment resulted in fasting hyperinsulinemia, glucose tolerance, and increased fat mass in the AIL rats. Despite this, BMD remained higher in the LFD control rats. Therefore, we propose that the AIL of GK and F344 rats is a model to test gene-diet interactions of complex metabolic disorders such as obesity-induced type-2 diabetes and osteoporosis.

DISCUSSION

Osteoporosis is characterized by compromised bone strength leading to an increased risk of fracture, and influenced by multiple genes and environmental factors. Identification of the genes underlying bone structure and strength will provide important insight regarding the genetics and mechanisms of osteoporosis and fracture risk. In human studies, it has proven difficult to find statistical linkage with genome-wide significance. Typically only suggestive linkage has been found, indicating that rather than a few genes with major effects, there are many genes each having a subtle effect on the overall bone phenotype. Replication studies has also proven to be difficult because the families/pedigrees/sib pairs between genome scans have differences in ethnicity, environmental factors, gender, age, etc. In addition, most genetic studies have focused on BMD, which only explains a part of the osteoporotic fracture risk.

Therefore, dissection of the genetic determinants of osteoporosis has been more effective in rat and mice models than in human populations due to improved control of environmental factors and minimised genetic heterogeneity.

Other advantages of using an animal model is the possibility to study clinically relevant phenotypes reflecting the ability of bone to resist fracture. Since collection of these phenotypes requires destructive testing of the bone, this is only possible in animal models. Rat models are less commonly used in genetic studies of bone, but offer advantages over mice because of larger bone size, allowing more precise structural and biomechanical measurements.

In this thesis, we characterized tibia for the majority of bone properties influencing bone strength and susceptibility to fracture in an F2 progeny of GK and F344 rats that concomitantly segregate type 2-like diabetes. All phenotypes were tested for genetic linkage and several chromosome regions linked to bone size, structure and strength were identified. Since the F2 progeny represented both males and females

from two reciprocal crosses with divergent mtDNA, we were also able to identify effects of both sex- and reciprocal cross on the genetic regulation of bone phenotypes. The observed interaction between nuclear QTLs for bone traits and reciprocal cross demonstrates a new and important aspect to be considered when interpreting the genetics of osteoporosis.

Reciprocal cross specific QTLs

Several QTLs were identified for bone phenotypes depending on the, most likely, mtDNA origin (haplotype) in both males and females and the subsequent likelihohood ratio tests confirmed a significant reciprocal cross interaction for twelve QTLs on chr 1, 2, 3, 4, 6, 10, 14, and 18. Interestingly, within these regions a number of nuclear encoded mitochondrial genes were found.

Reciprocal-cross setup has earlier been used, to understand the effect of maternal factors on various traits [112-113]. Other factors that are involved in the maternal inheritance of mitochondria include effect of the sex chromosomes, genomic imprinting and the maternal environment. Together these multiple factors make it complicated to identify genetic variations in nuclear encoded genes that affect complex diseases such as osteoporosis, and to estimate the contribution of each maternally inherited factor. However, the more than 100 variant positions that have been identified in the mtDNA of GK compared to F344 [97], support the involvement of mtDNA as a major factor behind the observed reciprocal cross effect in our studies.

Sex-specific QTLs

The sex-separated QTL analyses identified five female-specific QTLs linked to pQCT phenotypes on chr 1, 2, 3, 5 and 10 and one male specific QTL on chr 9 linked to endosteal area at fibula-site, measured by 3D CT. These results, lend further support to the notion that genes regulating bone phenotypes act in a sex-specific manner [45-46, 114-115].

Four different methods for bone analyses

By using four different methods i.e., DXA (bone mass), pQCT (trabecular and cortical bone), 3D CT (bone size) and biomechanical testing (bone strength) for bone analysis in the rats, we incorporated the majority of bone properties influencing bone strength and susceptibility to fracture.

The QTL mapping for all measured phenotypes identified specific chromosomal regions linked to bone size, structure or strength, illustrating the importance of analyzing different determinants that affect bone strength in order to detect candidate genes or pathways for different aspects of bone regulation, from the macro- to the micro-structural level. In addition, overlapping genetic regions were detected on chr 1, 3, 4, 6, 8, 10 and 14, suggesting pleiotropy. The co-localized QTLs on chr 4 and chr 6 were consistently detected in either sex and showed more significant linkage in one reciprocal cross depending on the mtDNA origin. To elucidate the presence of several QTLs in close proximity, compared to a single QTL affecting multiple phenotypes, additional studies are needed. Such studies could include strategies to increase the genetic resolution e.g. by increasing the sample size, increasing the number of intercross generations (generating advanced intercross lines) or breeding of small congenics.

Most interesting region and homology to humans

The most interesting region was identified on chr 1, with linkage to multiple bone strength related phenotypes in both males and females, and with predominately higher linkage in the cross with maternally inherited mtDNA from GK. Adding to the evidence that this is an important region for bone regulation is that this region overlaps previously identified QTLs linked to bone traits in other rat strains[77, 92] and includes the osteoporosis candidate genes transforming growth factor beta 1 (TGFB1) [62] and estrogen receptor alpha (ESR1) [53]that are mapped within this locus. The same region also showed linkage to fasting glucose [86], establishing this region as a candidate region for further studies to elucidate the possible genetic associations between bone-related phenotypes and traits affecting type-2 diabetes.

The GK and F344 rats

Due to the contradictory results that have been reported regarding the effects of diabetes-related phenotypes on bone, it is highly interesting to further explore the genetic regulation of bone in the presence of diabetes. Based on the observed characteristics showing skeletal changes between the GK and F344 rat in conjunction with type-2 diabetes, these rats are well suited as a model for such investigations. Overall, all measured bone phenotypes showed predominantely higher values in the diabetic GK rat compared to the non-diabetic F344 rat. Additionally, the GK alleles had an increasing effect on the genotypic mean values in the F2 for the majority of identified QTLs. These observations are in line with the reports showing that type-2 diabetes has anabolic effects on bone [12]. In addition, in order to develop more effective approaches to prevent and treat the diseases, it is relevant to delineate the genetic factors of these complex diseases in conjunction with environmental and lifestyle factors. To mimic the Western lifestyle associated with an overconsumption of dietary fat, both the F2-progeny and the AIL rats were fed modified fat enriched diets. In paper IV the experimentally design was to test the phenotypic responses of the high-fat diet on obesity-induced diabetes and osteoporosis. The outcome of the study showed that modifiable lifestyle factors such as a high-fat diet can significantly alter metabolic responses contributing to bone metabolism and diabetes related phenotypes, within a short intervention time. These rat strains therefore represent a suitable model for testing gene-diet interactions mimicking consequences of typically Westernized lifestyle with overconsumption of dietary fat.

Summary

In summary, we identified several chromosome regions linked to osteoporosis-related phenotypes including bone size, structure and strength in inbred GK and F344 F2 rats. Since the F2 progeny represented both males and females from two reciprocal crosses with divergent mtDNA, we were also able to identify effects of both sex- and reciprocal cross on the genetic regulation of bone phenotypes.

The most interesting region was identified on chromosome 1 with linkage to several bone phenotypes as well as fasting glucose. Therefore this region would be a strong candidate for a focused investigation to clarify possible shared mechanisms between genetic regulation of bone and diabetes related phenotypes and interaction with mitochondrial DNA. Yet, the chromosomal regions linked to each phenotype are very large, and likely to harbor several distinct sub-loci, and development of congenic strains are needed to isolate and narrow the identified regions.

In addition, we show that this animal model for co-segregating type-2 diabetes and bone phenotypes are well suited for studying the pathophysiology and the genetics for two increasingly common diseases.

CONCLUSIONS

From the papers included in this thesis it can be concluded that:

- By measuring the majority of bone properties influencing bone strength and susceptibility to fracture, several specific chromosomal regions for bone size, structure and strength were identified in an F2 rat progeny. These findings illustrate the importance of analyzing all determinants that affect bone strength to more accurately detect candidate genes for bone regulation.

- Since the F2 progeny represented both males and females from two reciprocal crosses with divergent mtDNA, we were also able to identify effects of both sex- and reciprocal cross on the genetic regulation of bone strength related phenotypes.

- The observed interaction between nuclear QTLs for bone traits and reciprocal cross could possibly be explained by mtDNA variation and demonstrates a new important aspect to be considered when interpreting the genetics of osteoporosis.

- The 3D CT method provided high precision measurements and can be used as a new tool for determining bone size characteristics in animal models.

- The most interesting region was identified on chr 1, with linkage to multiple bone strength related phenotypes in both males and females, and with predominately higher linkage in the cross with maternally inherited mtDNA from GK. The same region also showed linkage to fasting glucose, establishing this region as a strong candidate for a focused investigation to clarify possible shared genetic mechanisms between bone strength-related phenotypes and traits affecting type-2 diabetes and interaction with mitochondrial DNA.

- The characteristics of the GK and F344 rat strains, make these rats well suited for genetic dissection of traits affecting bone strength and the identification of genes related to skeletal fracture through pathways shared with type-2 diabetes. In

addition these rats are suitable for testing gene-diet interactions for two increasingly common diseases.

FUTURE PERSPECTIVES

The most interesting region was identified on chromosome 1 with linkage to several bone phenotypes as well as fasting glucose. Therefore this region would be a strong candidate for a focused investigation to clarify possible shared mechanisms between genetic regulation of bone and diabetes related phenotypes and interaction with mitochondrial DNA. The strategy would be to isolate and narrow the identified region by using congenic strains and/or AILs of GK and F344 rats. To confirm that the region on chromosome 1 could contain genes with mitochondrial interactions with importance for the pathogenesis of type-2 diabetes and osteoporosis, phenotyping including mitochondrial function, diabetic state and bone analysis in the congenics would be necessary.

Additionally, to further delineate the factors underlying the complex genetic architecture of bone phenotypes would involve searching for epistatic effects between the identified genetic loci that might contribute to fracture risk. Imprinted QTL analysis to provide additional evidence for mitochondrial interaction with nuclear QTLs, in a larger sample size would also be necessary.

The ultimate future goal would be to identify novel candidate genes implicated in both bone regulation and type-2 diabetes and eventually study the potential role of these genes in humans through population based association studies.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Osteoporos är en skelettsjukdom som karaktäriseras av reducerad benmassa vilket leder till en ökad risk för frakturer. Omkring hälften av alla kvinnor kommer någon gång efter 50 års ålder att drabbas av minst en osteoporosrelaterad fraktur. De viktigaste riskfaktorerna för fraktur är kön och ålder men även ärftliga faktorer och miljöfaktorer är av stor betydelse.

För att diagnostisera och identifiera patienter som löper risk att drabbas av osteoporos mäter man benmassa Dessa kan till ca 70 % förklaras av genetiska faktorer. Ärftlighet har studerats i familjer med osteoporos men kunskapen om vilka gener som påverkar risken att utveckla osteoporos är idag ofullständig. Att identifiera enskilda gener skulle bidra till ökad kunskap som kan användas för att utveckla nya och effektivare läkemedel mot osteoporos.

I denna avhandling (studie I-III) identifieras genregioner kopplade till benvävnadens struktur, hållfasthet och benstorlek i en råttmodell. Det finns flera fördelar med att tillämpa en råttmodell vid identifiering av gener som har betydelse för osteoporos: väl utvecklade mätmetoder finns tillgängliga för råttor där man med god precision och upplösning kan studera frakturrelaterade benfenotyper i tre dimensioner, möjligheten att inavla stammar vilket leder till minskat antal genvarianter samt att miljön kan hållas kontrollerad. Vi har använt en modell som bygger på en korsning mellan den diabetiska råttan GK och den icke-diabetiska F344 råttan som korsats i två generationer. Resultatet blev en F2 population bestående av 206 råttor som representerar båda könen samt har olika mitokondrie-DNA ursprung. GK råttan är en etablerad stam för genetiska studier om typ-2 diabetes men den har även använts för benstudier. Eftersom mycket pågående forskning rapporterar ökande bevis för en koppling mellan diabetes och benmetabolism är denna modell extra intressant.

Den genetiska analysen grundar sig på att man korsar en sjukdomsbenägen råttstam med en icke-sjukdomsbenägen råttstam. Avkomman kommer att ha en blandning av sjukdomsbenägna och icke-sjukdomsbenägna alleler (olika former av gener) och de kommer därför att visa en spridning i sjukdomsgrad. Varje råtta analyseras med DNA markörer (genotypdata) och karaktäriseras för sjukdomsspecifika egenskaper (fenotyper). Fenotyperna testas sedan för koppling till genotyper och genregioner kan identifieras genom *kopplingsanalys*. När en genregion har identifierats innehåller denna oftast flera hundra gener. För att se att genregionen verkligen innehåller kandidatgener med betydelse för osteoporos behöver man isolera genregionen genom avel i sk. *kongena* stammar.

En kongen råttstam uppnås genom korsning i flera omgångar tills man fått ett genfragment från den ena stammen på en bakgrund av den andra. Är denna genregion av betydelse för sjukdomen kommer den kongena råttan att ha en avvikande sjukdomsbenägenhet än råttor som saknar detta genfragment. Genom att minska denna genregion med nya korsningar samt genom att studera den fenotypiska effekten av denna genregion kan man slutligen identifiera kandidatgener för en specifik sjukdom. Informationen ligger sedan till grund för fortsatta studier i människa där homologa regioner kan identifieras, tack vare att genomet hos råtta och människa är väl kartlagt, och testas för funktion och association till eventuell sjukdom hos manniska.

I studie I-III karaktäriserades tibia (skenben) från F2 populationen med tre olika metoder; pQCT för trabekulär och kortikal benkvalitet, CT för benstorlek i olika dimensioner och benmineralinnehåll och biomekaniskt test för frakturbenägenhet. Alla erhållna frakturrelaterade fenotyper testades sedan för koppling till 192 genetiska markörer jämnt fördelade över hela genomet. Ett flertal genregioner identifierades i båda könen kopplat till både benstruktur, storlek och styrka. Några genregioner hittades bara i honor medan vissa i endast hanar vilket tyder på en könsspecifik genetisk reglering. Resultatet visade också olika genetisk koppling

beroende på vilket mitokondrie-DNA ursprung råttorna hade, antingen från GK eller F344. Eftersom mitokondrie-DNA ärvs endast från mamman bestämdes detta utifrån vilken ”urmoder” råttorna hade två generationer tillbaka i korsningsschemat. Den mest intressanta regionen identifierades på kromosom 1 och visade koppling till både benstruktur, storlek och styrka samt överlappade även genregioner som påverkar fasteglukos. Denna region skulle därför vara en stark kandidat för ytterligare karaktärisering med kongena stammar för att slutligen identifiera en eventuell gen/gener med betydelse för osteoporos med eventuell koppling till diabetes. Sammanfattningsvis visar våra resultat i studie I-III att den genetiska regleringen av benfenotyper är komplex. Exempelvis styrs trabekulärt och kortikalt ben av olika gener samt uttrycks olika bland honor och hanar. Även mitokondrie-DNA ursprung verkar ha betydelse men flera studier i större populationer behövs för att konfirmera detta.

I studie IV, visas även att en "advanced intercross line" (AIL) mellan GK och F344 råttor med fördel kan användas som en modell för att studera effekter av livsstilsfaktorer såsom fettrik diet på fenotyper relaterat till både ben- och energimetabolism. Denna modell som korsats i 20-21 generationer gör det möjligt att identifiera gener med högre upplösning än i F2 generationen och kan därför användas till att öka förståelsen kring de bakomliggande genetiska mekanismerna för diabetes och benmetabolism och deras samverkan.

ACKNOWLEDGEMENTS

Many people have contributed to the completion of this thesis in various ways. I would especially like to thank:

Kristina, my supervisor. Your contributions have been invaluable for the progress of this thesis. It has had many ups and downs but somehow you managed to get me back on track and encouraged me to finish this project. I admire you for being extremely productive and organized, for always being well-prepared in meetings and providing constructive ideas to progress the project. Thanks for sharing your outstanding skills in clinical research and for always having time for discussions when needed, in spite of your busy schedule.

Holger, my co-supervisor. Thanks for introducing me to rat genetics, and for sharing your outstanding knowledge in genetics and many other interesting fields. I am fascinated by your dedicated engagement for scientific research and for always having new enthusiastic ideas in mind.

Fiona, co-author and "mental coach". There are not words enough to thank you. You are absolutely fantastic and without your help and support this thesis would never have been completed. I really appreciate your help in the final preparation of this thesis but also for reading and correcting my long "swenglish" sentences in my manuscripts. Thanks also for being encouraging and caring by sharing small words of wisdom every now and then during the progress of this thesis, it has truly meant a lot to me!

Maria, co-supervisor, co-author and roommate. You give me so much energy and inspiration! I am very thankful for your involvement in the final stage of this thesis. Thanks for sharing your knowledge in rat genetics and for giving me important feed-back in general for my manuscripts. Thanks also for your good company and for all our amusing conversations about life in general.

All other co-authors, especially **Hee-bok Park** for spending many hours helping me with the statistical calculations and for sharing your knowledge and skills in general. **Mats Nilsson** for spending much time outside working hours helping me with bone analyses, always with great enthusiasm, in spite of your busy schedule. Outside our department, I would like to thank **Claes Ohlsson** and **Marc Grynpas** for your collaboration letting us use the bone analysis facilities at your departments. Special thanks to **Annette Hansevi, Luisa Moreno** and **Stephanie Neufeld** for performing the measurements.

All persons involved in the research group meetings at orthopedics, thanks everyone for giving me feed-back on my project and thanks for sharing your own results from the clinical research world, it has been truly inspiring and educative!

I also would like to thank three very important persons that have contributed to a very pleasant atmosphere in the orthopedic building, **Åsa**, **Lisbeth** and **Carin**, what a fantastic trio! Warmhearted and with such a good sense of humour. Thanks, for all nice conversations about life in general and for your endless support over the years. **Carin**, thanks also for helping me with administrative work. Former and present members of the "Thursday breakfast club", thanks all of you for lighting up Thursday mornings by contributing to many lively discussions filled with many laughs.

Lisa, my roommate at CRC, thanks for sharing your knowledge in the lab and for your good company and for all delicious cakes you have served us over the years.

Jitender, my other roommate at CRC, thanks for reading an correcting my thesis in the final preparations.

Rolf, thanks for always being helpful in fixing computer-related problems.

Other colleagues, that no longer work at the department but still very important for the progress of this thesis and for creating a nice and friendly atmosphere: **Emma**, my very first room mate at orthopedics, thanks for your company and for being a good and supportive friend. **Kaisa**, you have always inspired and fascinated me in

many ways, both in research but also as a friend and thanks for all nice Marimekko gifts. **Avinash**, for always being nice and helpful.

Slutligen, vill jag tacka alla mina nära och kära som fyller mitt liv utanför forskarvärlden med mycket glädje. **Annika**, tack för alla härliga luncher som vi avnjutit ihop, särskilt de på Simrishamnsgatan i vårsolen! **Sara**, **Louise** och **Zorica**, jag ser fram mot att fira flera 10-års jubileum med er! Ni är helt underbara! Tack alla ni andra i Helsingborg men också ni som bor lite längre bort. **Sofia**, jag letar ständigt efter ett passande hus till er i Skåne, när är ni redo att lämna östkusten? **Maria**, tack för all barnpassningshjälp, och för att ni är de bästa grannar man kan ha.

Min familj! Tack **mamma** och **pappa** för att ni har stöttat mig hela vägen fram till denna avhandling. Tack också mamma för alla mil du rest för att agera mormor till dina morgonpigga barnbarn ☺, din hjälp har varit ovärderlig!

Min kära syster **Sara**, tack för alla uppmuntrande och givande pratstunder från andra sidan Atlanten, tur att det finns Skype annars hade vi nog varit ruinerade! Storebror **Christoffer**, du är den mest ambitiösa personen jag känner samtidigt som du alltid är närvarande för dina barn, det beundrar jag dig verkligen för!

Sist men absolut inte minst, vill jag tacka de viktigaste personerna i mitt liv. **Martin**, vad hade jag gjort utan dig? Tack för att du alltid är så lugn och kärleksfull och påminner mig om vad som är viktigt i livet! **Ville**, du är en sann glädjespridare som alltid får mig på så gott humör, tack för alla ljuvliga stunder jag har fått dela med dig så långt i livet och för att du är en fantastisk storebror.
Alice och **Thea**, snacka om överraskning i dubbel bemärkelse! Ni är båda helt ljuvliga och jag är oerhört tacksam som får dela livets goda med er ♥.

REFERENCES

[1] Rossert JadC, B. Type I Collagen: Structure, Synthesis, and Regulation. In: Bilezikian JP RLaRG, editor. Principles of Bone Biology. San Diego: Academic press; 1996.

[2] Marks SaH, DC. The Structure and Development of Bone. In: Bilezikian JP RLaRG, editor. Principles of Bone Biology. San Diego: Academic press; 1996.

[3] Downey PA, Siegel MI. Bone biology and the clinical implications for osteoporosis. Phys Ther 2006;86: 77-91.

[4] Baron R. General principles of bone biology 5th ed. Philadelphia, USA: Lippinicott, Williams and Wilkins; 2003.

[5] WHO. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. In: Report of a WHO study group.: World Health Organ Tech Rep Ser; 1994, p. 1-129.

[6] Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmo. Osteoporos Int 2000;11: 669-74.

[7] Woolf A, Akesson, K. Osteoporosis. In: Atlas of Investigation & Management. Oxford: Clinical Publishing; 2008.

[8] Rodan G. Pathophysiology of osteoporosis. In: Bilezikian JP RLaRG, editor. Principles of bone biology. San Diego, USA: Academic Press; 1996, p. 979-990.

[9] Espallargues M, Sampietro-Colom L, Estrada MD, Sola M, del Rio L, Setoain J, Granados A. Identifying bone-mass-related risk factors for fracture to guide bone densitometry measurements: a systematic review of the literature. Osteoporos Int 2001;12: 811-22.

[10] Kayath MJ, Tavares EF, Dib SA, Vieira JG. Prospective bone mineral density evaluation in patients with insulin-dependent diabetes mellitus. J Diabetes Complications 1998;12: 133-9.

[11] Tuominen JT, Impivaara O, Puukka P, Ronnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. Diabetes Care 1999;22: 1196-200.

[12] Holmberg AH, Nilsson PM, Nilsson JA, Akesson K. The association between hyperglycemia and fracture risk in middle age. A prospective, population-based study of 22,444 men and 10,902 women. J Clin Endocrinol Metab 2008;93: 815-22.

[13] Liefde I, van der Klift M, de Laet CE, van Daele PL, Hofman A, Pols HA. Bone mineral density and fracture risk in type-2 diabetes mellitus: the Rotterdam Study. Osteoporos Int 2005;16: 1713-20.

[14] Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 2002;359: 1929-36.

[15] Melton LJ, 3rd. Adverse outcomes of osteoporotic fractures in the general population. J Bone Miner Res 2003;18: 1139-41.

[16] Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9: 1137-41.

[17] Faulkner K. Clinical use of bone densitometry. In: Marcus R FD, Kelsey J, editor. Osteoporosis. 2nd ed. San Diego, USA: Academic press; 2001, p. 433-58.

[18] Genant HK, Gordon C, Jiang Y, Link TM, Hans D, Majumdar S, Lang TF. Advanced imaging of the macrostructure and microstructure of bone. Horm Res 2000;54 Suppl 1: 24-30.

[19] Kanis J. Assessment of fracture risk. Who should be screened? In: MJ F, editor. Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 5th ed. Philadelphia, USA: Lippinicott, Williams and Wilkins; 2003, p. 316-323.

[20] Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A. Case finding for the management of osteoporosis with FRAX--

assessment and intervention thresholds for the UK. Osteoporos Int 2008;19: 1395-408.

[21] Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet 1996;348: 1535-41.

[22] Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001;409: 928-33.

[23] Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D,

McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. Science 2001;291: 1304-51.

[24] Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999;22: 231-8.

[25] Livingston RJ, von Niederhausern A, Jegga AG, Crawford DC, Carlson CS, Rieder MJ, Gowrisankar S, Aronow BJ, Weiss RB, Nickerson DA. Pattern of sequence variation across 213 environmental response genes. Genome Res 2004;14: 1821-31.

[26] Rapp JP. Genetic analysis of inherited hypertension in the rat. Physiol Rev 2000;80: 135-72.

[27] Morahan G, Morel L. Genetics of autoimmune diseases in humans and in animal models. Curr Opin Immunol 2002;14: 803-11.

[28] Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11: 241-7.

[29] Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. Genetics 1994;138: 963-71.

[30] Fakhrai-Rad H, Nikoshkov A, Kamel A, Fernstrom M, Zierath JR, Norgren S, Luthman H, Galli J. Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats. Hum Mol Genet 2000;9: 2149-58.

[31] Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V, Luthman H, Renstrom E. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science 2010;327: 217-20.

[32] Swanberg M, Lidman O, Padyukov L, Eriksson P, Akesson E, Jagodic M, Lobell A, Khademi M, Borjesson O, Lindgren CM, Lundman P, Brookes AJ, Kere J, Luthman H, Alfredsson L, Hillert J, Klareskog L, Hamsten A, Piehl F, Olsson T. MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. Nat Genet 2005;37: 486-94.

[33] Smith DM, Nance WE, Kang KW, Christian JC, Johnston CC, Jr. Genetic factors in determining bone mass. J Clin Invest 1973;52: 2800-8.

[34] Dequeker J, Nijs J, Verstraeten A, Geusens P, Gevers G. Genetic determinants of bone mineral content at the spine and radius: a twin study. Bone 1987;8: 207-9.

[35] Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987;80: 706-10.

[36] Koller DL, Liu G, Econs MJ, Hui SL, Morin PA, Joslyn G, Rodriguez LA, Conneally PM, Christian JC, Johnston CC, Jr., Foroud T, Peacock M. Genome screen for quantitative trait loci underlying normal variation in femoral structure. J Bone Miner Res 2001;16: 985-91.

[37] Flicker L, Faulkner KG, Hopper JL, Green RM, Kaymacki B, Nowson CA, Young D, Wark JD. Determinants of hip axis length in women aged 10-89 years: a twin study. Bone 1996;18: 41-5.

[38] Arden NK, Baker J, Hogg C, Baan K, Spector TD. The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. J Bone Miner Res 1996;11: 530-4.

[39] Beamer WG, Donahue LR, Rosen CJ, Baylink DJ. Genetic variability in adult bone density among inbred strains of mice. Bone 1996;18: 397-403.

[40] Deng HW, Xu FH, Huang QY, Shen H, Deng H, Conway T, Liu YJ, Liu YZ, Li JL, Zhang HT, Davies KM, Recker RR. A whole-genome linkage scan suggests several genomic regions potentially containing quantitative trait Loci for osteoporosis. J Clin Endocrinol Metab 2002;87: 5151-9.

[41] Kammerer CM, Schneider JL, Cole SA, Hixson JE, Samollow PB, O'Connell JR, Perez R, Dyer TD, Almasy L, Blangero J, Bauer RL, Mitchell BD. Quantitative trait loci on chromosomes 2p, 4p, and 13q influence bone mineral density of the forearm and hip in Mexican Americans. J Bone Miner Res 2003;18: 2245-52.

[42] Karasik D, Myers RH, Cupples LA, Hannan MT, Gagnon DR, Herbert A, Kiel DP. Genome screen for quantitative trait loci contributing to normal variation in bone mineral density: the Framingham Study. J Bone Miner Res 2002;17: 1718-27.

[43] Styrkarsdottir U, Cazier JB, Kong A, Rolfsson O, Larsen H, Bjarnadottir E, Johannsdottir VD, Sigurdardottir MS, Bagger Y, Christiansen C, Reynisdottir I, Grant SF, Jonasson K, Frigge ML, Gulcher JR, Sigurdsson G, Stefansson K. Linkage of osteoporosis to chromosome 20p12 and association to BMP2. PLoS Biol 2003;1: E69.

[44] Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M, Prince RL, Thompson D, Thompson E, Bailey M, Kleyn PW, Sambrook P, Shi MM, Spector TD. Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. Am J Hum Genet 2003;72: 144-55.

[45] Ioannidis JP, Ng MY, Sham PC, Zintzaras E, Lewis CM, Deng HW, Econs MJ, Karasik D, Devoto M, Kammerer CM, Spector T, Andrew T, Cupples LA, Duncan EL, Foroud T, Kiel DP, Koller D, Langdahl B, Mitchell BD, Peacock M, Recker R, Shen H, Sol-Church K, Spotila LD, Uitterlinden AG, Wilson SG, Kung AW, Ralston SH. Meta-analysis of

genome-wide scans provides evidence for sex- and site-specific regulation of bone mass. J Bone Miner Res 2007;22: 173-83.

[46] Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ. Sex-specific quantitative trait loci contribute to normal variation in bone structure at the proximal femur in men. Bone 2005;37: 467-73.

[47] Ioannidis JP. Genetic associations: false or true? Trends Mol Med 2003;9: 135-8.

[48] Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367: 284-7.

[49] Uitterlinden AG, Ralston SH, Brandi ML, Carey AH, Grinberg D, Langdahl BL, Lips P, Lorenc R, Obermayer-Pietsch B, Reeve J, Reid DM, Amedei A, Bassiti A, Bustamante M, Husted LB, Diez-Perez A, Dobnig H, Dunning AM, Enjuanes A, Fahrleitner-Pammer A, Fang Y, Karczmarewicz E, Kruk M, van Leeuwen JP, Mavilia C, van Meurs JB, Mangion J, McGuigan FE, Pols HA, Renner W, Rivadeneira F, van Schoor NM, Scollen S, Sherlock RE, Ioannidis JP. The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. Ann Intern Med 2006;145: 255-64.

[50] Tenne M, McGuigan F, Jansson L, Gerdhem P, Obrant KJ, Luthman H, Akesson K. Genetic variation in the PTH pathway and bone phenotypes in elderly women: evaluation of PTH, PTHLH, PTHR1 and PTHR2 genes. Bone 2008;42: 719-27.

[51] Masi L, Becherini L, Colli E, Gennari L, Mansani R, Falchetti A, Becorpi AM, Cepollaro C, Gonnelli S, Tanini A, Brandi ML. Polymorphisms of the calcitonin receptor gene are associated with bone mineral density in postmenopausal Italian women. Biochem Biophys Res Commun 1998;248: 190-5.

[52] Young R, Wu F, Van de Water N, Ames R, Gamble G, Reid IR. Calcium sensing receptor gene A986S polymorphism and responsiveness to calcium supplementation in postmenopausal women. J Clin Endocrinol Metab 2003;88: 697-700.

[53] Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM, Schuit SC, Sherlock RE, Uitterlinden AG. Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. Jama 2004;292: 2105-14.

[54] Tofteng CL, Kindmark A, Brandstrom H, Abrahamsen B, Petersen S, Stiger F, Stilgren LS, Jensen JE, Vestergaard P, Langdahl BL, Mosekilde L. Polymorphisms in the CYP19 and AR genes--relation to bone mass and longitudinal bone changes in postmenopausal women with or without hormone replacement therapy: The Danish Osteoporosis Prevention Study. Calcif Tissue Int 2004;74: 25-34.

[55] Mann V, Ralston SH. Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. Bone 2003;32: 711-7.

[56] Gerdhem P, Brandstrom H, Stiger F, Obrant K, Melhus H, Ljunggren O, Kindmark A, Akesson K. Association of the collagen type 1 (COL1A 1) Sp1 binding site polymorphism to femoral neck bone mineral density and wrist fracture in 1044 elderly Swedish women. Calcif Tissue Int 2004;74: 264-9.

[57] Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, Aspden RM, Ralston SH. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. J Clin Invest 2001;107: 899-907.

[58] Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, Lorenc R, Obermayer-Pietsch B, Scollen S, Bustamante M, Husted LB, Carey AH, Diez-Perez A, Dunning AM, Falchetti A, Karczmarewicz E, Kruk M, van Leeuwen JP, van Meurs JB, Mangion J, McGuigan FE, Mellibovsky L, del Monte F, Pols HA, Reeve J, Reid DM, Renner W, Rivadeneira F, van Schoor NM, Sherlock RE, Ioannidis JP. Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. PLoS Med 2006;3: e90.

[59] Rivadeneira F, van Meurs JB, Kant J, Zillikens MC, Stolk L, Beck TJ, Arp P, Schuit SC, Hofman A, Houwing-Duistermaat JJ, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG. Estrogen receptor beta (ESR2)

polymorphisms in interaction with estrogen receptor alpha (ESR1) and insulin-like growth factor I (IGF1) variants influence the risk of fracture in postmenopausal women. J Bone Miner Res 2006;21: 1443-56.

[60] Nordstrom A, Gerdhem P, Brandstrom H, Stiger F, Lerner UH, Lorentzon M, Obrant K, Nordstrom P, Akesson K. Interleukin-6 promoter polymorphism is associated with bone quality assessed by calcaneus ultrasound and previous fractures in a cohort of 75-year-old women. Osteoporos Int 2004;15: 820-6.

[61] McGuigan FE, Larzenius E, Callreus M, Gerdhem P, Luthman H, Akesson K. Variation in the BMP2 gene: bone mineral density and ultrasound in young adult and elderly women. Calcif Tissue Int 2007;81: 254-62.

[62] Langdahl BL, Uitterlinden AG, Ralston SH, Trikalinos TA, Balcells S, Brandi ML, Scollen S, Lips P, Lorenc R, Obermayer-Pietsch B, Reid DM, Armas JB, Arp PP, Bassiti A, Bustamante M, Husted LB, Carey AH, Perez Cano R, Dobnig H, Dunning AM, Fahrleitner-Pammer A, Falchetti A, Karczmarewicz E, Kruk M, van Leeuwen JP, Masi L, van Meurs JB, Mangion J, McGuigan FE, Mellibovsky L, Mosekilde L, Nogues X, Pols HA, Reeve J, Renner W, Rivadeneira F, van Schoor NM, Ioannidis JP. Large-scale analysis of association between polymorphisms in the transforming growth factor beta 1 gene (TGFB1) and osteoporosis: the GENOMOS study. Bone 2008;42: 969-81.

[63] 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: 866-75.

[64] Uitterlinden AG, Arp PP, Paeper BW, Charmley P, Proll S, Rivadeneira F, Fang Y, van Meurs JB, Britschgi TB, Latham JA, Schatzman RC, Pols HA, Brunkow ME. Polymorphisms in the sclerosteosis/van Buchem disease gene (SOST) region are associated with bone-mineral density in elderly whites. Am J Hum Genet 2004;75: 1032-45.

[65] Ichikawa S, Koller DL, Johnson ML, Lai D, Xuei X, Edenberg HJ, Klein RF, Orwoll ES, Hui SL, Foroud TM, Peacock M, Econs MJ. Human ALOX12, but not ALOX15, is associated with BMD in white men and women. J Bone Miner Res 2006;21: 556-64.

[66] Kim JG, Kim JH, Kim JY, Ku SY, Jee BC, Suh CS, Kim SH, Choi YM. Association between osteoprotegerin (OPG), receptor activator of nuclear factor-kappaB (RANK), and RANK ligand (RANKL) gene polymorphisms and circulating OPG, soluble RANKL levels, and bone mineral density in Korean postmenopausal women. Menopause 2007;14: 913-8.

[67] Bouxsein ML, Uchiyama T, Rosen CJ, Shultz KL, Donahue LR, Turner CH, Sen S, Churchill GA, Muller R, Beamer WG. Mapping quantitative trait loci for vertebral trabecular bone volume fraction and microarchitecture in mice. J Bone Miner Res 2004;19: 587-99.

[68] Beamer WG, Shultz KL, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ, Donahue LR. Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. Mamm Genome 1999;10: 1043-9.

[69] 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: 1648-56.

[70] Lang DH, Sharkey NA, Mack HA, Vogler GP, Vandenbergh DJ, Blizard DA, Stout JT, McClearn GE. Quantitative trait loci analysis of structural and material skeletal phenotypes in C57BL/6J and DBA/2 second-generation and recombinant inbred mice. J Bone Miner Res 2005;20: 88-99.

[71] Masinde GL, Li X, Gu W, Wergedal J, Mohan S, Baylink DJ. Quantitative trait loci for bone density in mice: the genes determining total skeletal density and femur density show little overlap in F2 mice. Calcif Tissue Int 2002;71: 421-8.

[72] Masinde GL, Wergedal J, Davidson H, Mohan S, Li R, Li X, Baylink DJ. Quantitative trait loci for periosteal circumference (PC): identification of single loci and epistatic effects in F2 MRL/SJL mice. Bone 2003;32: 554-60.

[73] Volkman SK, Galecki AT, Burke DT, Paczas MR, Moalli MR, Miller RA, Goldstein SA. Quantitative trait loci for femoral size and shape in a genetically heterogeneous mouse population. J Bone Miner Res 2003;18: 1497-505.

[74] Volkman SK, Galecki AT, Burke DT, Miller RA, Goldstein SA. Quantitative trait loci that modulate femoral mechanical properties in a genetically heterogeneous mouse population. J Bone Miner Res 2004;19: 1497-505.

[75] Wergedal JE, Sheng MH, Ackert-Bicknell CL, Beamer WG, Baylink DJ. Mouse genetic model for bone strength and size phenotypes: NZB/B1NJ and RF/J inbred strains. Bone 2002;31: 670-4.

[76] Alam I, Sun Q, Liu L, Koller DL, Carr LG, Econs MJ, Foroud T, Turner CH. Sex-specific genetic loci for femoral neck bone mass and strength identified in inbred COP and DA rats. J Bone Miner Res 2008;23: 850-9.

[77] Koller DL, Alam I, Sun Q, Liu L, Fishburn T, Carr LG, Econs MJ, Foroud T, Turner CH. Genome screen for bone mineral density phenotypes in Fisher 344 and Lewis rat strains. Mamm Genome 2005;16: 578-86.

[78] Klein RF, Allard J, Avnur Z, Nikolcheva T, Rotstein D, Carlos AS, Shea M, Waters RV, Belknap JK, Peltz G, Orwoll ES. Regulation of bone mass in mice by the lipoxygenase gene Alox15. Science 2004;303: 229-32.

[79] Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ, Rosen CJ. Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. J Bone Miner Res 2001;16: 1195-206.

[80] Orwoll ES, Belknap JK, Klein RF. Gender specificity in the genetic determinants of peak bone mass. J Bone Miner Res 2001;16: 1962-71.

[81] Turner CH, Roeder RK, Wieczorek A, Foroud T, Liu G, Peacock M. Variability in skeletal mass, structure, and biomechanical properties among inbred strains of rats. J Bone Miner Res 2001;16: 1532-9.

[82] Goto Y, Kakizaki M, Masaki N. Production of spontaneous diabetic rats by repetition of selective breeding. Tohoku J Exp Med 1976;119: 85-90.

[83] Bisbis S, Bailbe D, Tormo MA, Picarel-Blanchot F, Derouet M, Simon J, Portha B. Insulin resistance in the GK rat: decreased receptor

number but normal kinase activity in liver. Am J Physiol 1993;265: E807-13.

[84] Kimura K, Toyota T, Kakizaki M, Kudo M, Takebe K, Goto Y. Impaired insulin secretion in the spontaneous diabetes rats. Tohoku J Exp Med 1982;137: 453-9.

[85] Portha B, Serradas P, Bailbe D, Suzuki K, Goto Y, Giroix MH. Beta-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes 1991;40: 486-91.

[86] Galli J, Li LS, Glaser A, Ostenson CG, Jiao H, Fakhrai-Rad H, Jacob HJ, Lander ES, Luthman H. Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. Nat Genet 1996;12: 31-7.

[87] Gauguier D, Froguel P, Parent V, Bernard C, Bihoreau MT, Portha B, James MR, Penicaud L, Lathrop M, Ktorza A. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. Nat Genet 1996;12: 38-43.

[88] Ahmad T, Ohlsson C, Ostenson CG, Kreicbergs A. Peripheral quantitative computed tomography for the detection of diabetic osteopathy: a study in the Goto-Kakizaki rat. Invest Radiol 2003;38: 171-6.

[89] Ahmad T, Ohlsson C, Saaf M, Ostenson CG, Kreicbergs A. Skeletal changes in type-2 diabetic Goto-Kakizaki rats. J Endocrinol 2003;178: 111-6.

[90] Ostenson CG, Fiere V, Ahmed M, Lindstrom P, Brismar K, Brismar T, Kreicbergs A. Decreased cortical bone thickness in spontaneously non-insulin-dependent diabetic GK rats. J Diabetes Complications 1997;11: 319-22.

[91] Alam I, Sun Q, Liu L, Koller DL, Fishburn T, Carr LG, Econs MJ, Foroud T, Turner CH. Whole-genome scan for linkage to bone strength and structure in inbred Fischer 344 and Lewis rats. J Bone Miner Res 2005;20: 1589-96.

[92] Alam I, Sun Q, Liu L, Koller DL, Fishburn T, Carr LG, Econs MJ, Foroud T, Turner CH. Identification of a quantitative trait locus on rat

chromosome 4 that is strongly linked to femoral neck structure and strength. Bone 2006;39: 93-9.

[93] Koller DL, Liu L, Alam I, Sun Q, Econs MJ, Foroud T, Turner CH. Epistatic effects contribute to variation in BMD in Fischer 344 x Lewis F2 rats. J Bone Miner Res 2008;23: 41-7.

[94] Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci U S A 1980;77: 6715-9.

[95] Ingman M, Kaessmann H, Paabo S, Gyllensten U. Mitochondrial genome variation and the origin of modern humans. Nature 2000;408: 708-13.

[96] Pravenec M, Hyakukoku M, Houstek J, Zidek V, Landa V, Mlejnek P, Miksik I, Dudova-Mothejzikova K, Pecina P, Vrbacky M, Drahota Z, Vojtiskova A, Mracek T, Kazdova L, Oliyarnyk O, Wang J, Ho C, Qi N, Sugimoto K, Kurtz T. Direct linkage of mitochondrial genome variation to risk factors for type 2 diabetes in conplastic strains. Genome Res 2007;17: 1319-26.

[97] Schlick NE, Jensen-Seaman MI, Orlebeke K, Kwitek AE, Jacob HJ, Lazar J. Sequence analysis of the complete mitochondrial DNA in 10 commonly used inbred rat strains. Am J Physiol Cell Physiol 2006;291: C1183-92.

[98] Delmas PD. Biochemical markers of bone turnover for the clinical investigation of osteoporosis. Osteoporos Int 1993;3 Suppl 1: 81-6.

[99] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130: 456-69.

[100] Nilsson M, Johnell O, Jonsson K, Redlund-Johnell I. Quantitative computed tomography in measurement of vertebral trabecular bone mass. A modified method. Acta Radiol 1988;29: 719-25.

[101] Jamsa T, Jalovaara P, Peng Z, Vaananen HK, Tuukkanen J. Comparison of three-point bending test and peripheral quantitative

computed tomography analysis in the evaluation of the strength of mouse femur and tibia. Bone 1998;23: 155-61.

[102] Turner CH. Biomechanics of bone: determinants of skeletal fragility and bone quality. Osteoporos Int 2002;13: 97-104.

[103] Fakhrai-Rad H, Jiao H, Li LS, Glaser A, Koike G, Jacob HJ, Luthman H, Galli J. A rat genetic linkage map including 67 new microsatellite markers. Mamm Genome 1999;10: 1102-5.

[104] Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1987;1: 174-81.

[105] Broman KW, Sen S, Owens SE, Manichaikul A, Southard-Smith EM, Churchill GA. The X chromosome in quantitative trait locus mapping. Genetics 2006;174: 2151-8.

[106] Manly KF, Cudmore RH, Jr., Meer JM. Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 2001;12: 930-2.

[107] Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265: 2037-48.

[108] Manly KF, Olson JM. Overview of QTL mapping software and introduction to map manager QT. Mamm Genome 1999;10: 327-34.

[109] Weiss LA, Pan L, Abney M, Ober C. The sex-specific genetic architecture of quantitative traits in humans. Nat Genet 2006;38: 218-22.

[110] Lynch M, Walsh B. Genetics and Analysis of Quantitative Traits. In. Sunderland, MA, USA: Sinauer Associates, Inc; 1998.

[111] Li L. Molecular genetics of type 2 diabetes. In: Department of Molecular Medicine. Stockholm: Karolinska Institutet; 2002. Thesis.

[112] Klindt J, Maurer RR. Reciprocal cross effects on growth hormone and prolactin secretion in cattle: influence of genotype and maternal environment. J Anim Sci 1986;62: 1660-5.

[113] Gilliam DM, Mantle MA, Barkhausen DA, Tweden DR. Effects of acute prenatal ethanol administration in a reciprocal cross of C57BL/6J and short-sleep mice: maternal effects and nonmaternal factors. Alcohol Clin Exp Res 1997;21: 28-34.

[114] Peacock M, Koller DL, Fishburn T, Krishnan S, Lai D, Hui S, Johnston CC, Foroud T, Econs MJ. Sex-specific and non-sex-specific quantitative trait loci contribute to normal variation in bone mineral density in men. J Clin Endocrinol Metab 2005;90: 3060-6.

[115] Ralston SH, Galwey N, MacKay I, Albagha OM, Cardon L, Compston JE, Cooper C, Duncan E, Keen R, Langdahl B, McLellan A, O'Riordan J, Pols HA, Reid DM, Uitterlinden AG, Wass J, Bennett ST. Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. Hum Mol Genet 2005;14: 943-51.

APPENDIX A

Table 1A. 2D DXA results of male GK, F344, GKxF344 F1 and F344xGK F1 rats.

Male	GK	F344	% Difference (p-value)	GK x F344 F1	F344 x GK F1	% Difference (p-value)
Number of rats	9	10		8	9	
Age (days)	231	229	NS	216	233	0.0003
DXA Parameters						
BMC (g)	10.8 ± 1.0	9.3 ± 0.4	16 (0.0003)	12.7 ± 1.1	11.9 ± 0.9	6.7 (NS)
BMD (g/cm^2)	0.32 ± 0.007	0.31 ± 0.007	3.2 (0.01)	0.33 ± 0.02	0.33 ± 0.01	~0 (NS)
Area (cm^2)	33.6 ± 2.5	29.7 ± 1.3	13 (0.0005)	38.1 ± 2.2	36.3 ± 2.1	5.0 (NS)
Tissue mass (g)	431 ± 31	424 ± 23	1.7 (NS)	512 ± 29	517 ± 35	-1.0 (NS)
Lean mass (g)	352 ± 18	319 ± 14	10 (0.0003)	404 ± 18	384 ± 20	5.2 (0.05)
Fat mass (%)	18.2 ± 4.9	24.6 ±3 .2	-6.4 (0.003)	21.1 ± 2.7	25.6 ± 2.7	-4.5 (0.004)

Phenotypes are uncorrected and presented as mean ± sd.

The difference between (GK –F344 and GKxF344F1-F344xGKxF1) are indicated, and nominal *p*-values determined by ANOVA are given.

Table 1B. 2D DXA results of female GK, F344, GKxF344 F1 and F344xGK F1 rats.

Female	GK	F344	% Difference (p-value)	GK x F344 F1	F344 x GK F1	% Difference (p-value)
Number of rats	10	10		10	10	
Age (days)	228	231	NS	212	236	(<10^{-}4)
DXA Parameters						
BMC (g)	8.3 ± 0.8	5.3 ± 0.5	55 (<10^{-}4)	6.9 ± 0.32	6.5 ± 0.6	6.1 (0.04)
BMD (g/cm^2)	0.31 ± 0.01	0.29 ± 0.01	6.9 (<10^{-}4)	0.30 ± 0.008	0.29 ± 0.01	3.4 (NS)
Area (cm^2)	26.3 ± 2.1	18.6 ± 1.32	41 (<10^{-}4)	23.4 ± 0.8	22.2 ± 1.4	5.4 (0.03)
Tissue mass (g)	304 ± 25	245 ± 15	24 (<10^{-}4)	257 ± 11	270 ± 10	-4.8 (0.02)
Lean mass (g)	250 ± 20	192 ± 12	30 (<10^{-}4)	221 ± 4.6	220 ± 9.4	0.5 (NS)
Fat mass (%)	17.9 ± 2.8	21.7 ± 3.6	-3.8 (0.02)	13.8 ± 2.8	18.2 ± 2.2	-4.4 (0.001)

Phenotypes are uncorrected and presented as mean ± sd.

The difference between (GK –F344 and GKxF344F1-F344xGKxF1) are indicated, and nominal *p*-values determined by ANOVA are given.

Table 2A. 3D CT results of male GK, F344, GKxF344 F1 and F344xGK F1 rats.

Male	GK	F344	% Difference (p-value)	GK x F344 F1	F344 x GK F1	% Difference (p-value)
Number of rats	9	10		8	9	
Age (days)	231	229	NS	216	233	0.0003
Bone size (Femur)						
TotBV (mm^3)	533 ± 42	519 ± 34	27 (NS)	620 ± 48	603 ± 65	2.8 (NS)
CortBV (mm^3)	193 ± 16	171 ± 14	13 (0.006)	207 ± 24	206 ± 26	0.5 (NS)
CortBV/TotBV (%)	36 ± 0.5	33 ± 2.0	3.0 (<10-4)	33 ± 1.0	34 ± 1.0	-1.0 (NS)
Length S (mm)	39.4 ± 0.9	40.1 ± 1.0	-1.7 (NS)	41.7 ± 0.7	41.3 ± 1.4	1.0 (NS)
Length C (mm)	44.3 ± 0.7	46.0 ± 2.6	-3.7 (NS)	48.0 ± 1.4	47.3 ± 2.7	1.5 (NS)
PAmid (mm^2)	17.6 ± 1.9	13.7 ± 1.6	28 (0.0002)	16.6 ± 1.2	16.1 ± 2.0	3.1 (NS)
EAmid (mm^2)	4.8 ± 0.8	3.4 ± 0.8	41 (0.0006)	4.3 ± 0.6	4.2 ± 1.3	2.4 (NS)
CortCSA (mm^2)	12.8 ± 1.3	10.3 ± 1.0	19 (0.0002)	12.3 ± 1.5	11.9 ± 1.3	3.4 (NS)
TotvBMC (mg/cm^3)	1330 ± 12	1316 ± 21	1.1 (NS)	1319 ± 18	1334 ± 14	-1.1 (NS)
CortvBMC (mg/cm^3)	1916 ± 8.0	1854 ± 27	3.3 (<10-4)	1872 ± 15	1877 ± 6.1	-0.3 (NS)

Phenotypes are uncorrected and presented as mean ± sd.

The difference between (GK –F344 and GKxF344F1-F344xGKxF1) are indicated, and nominal *p*-values determined by ANOVA are given.

Table 2B. 3D CT results of male GK, F344, GKxF344 F1 and F344xGK F1rats.

Female	**GK**	**F344**	**% Difference (p-value)**	**GK x F344 F1**	**F344 x GK F1**	**% Difference (p-value)**
Number of rats	10	10		10	10	
Age (days)	228	231	NS	212	236	(<10-4)
Bone size (Femur)						
TotBV (mm^3)	430 ± 18	337 ± 29	28 (<10-4)	400 ± 15	391 ± 22	2.3 (NS)
CortBV (mm^3)	151 ± 8.1	98 ± 6.0	54 (<10-4)	120 ± 4.4	120 ± 12	~0 (NS)
CortBV/TotBV (%)	35 ± 1.0	29 ± 3.0	6.0 (<10-4)	30 ± 1.0	31 ± 1.0	-1.0 (NS)
Length S (mm)	35.1 ± 0.6	33.5 ± 1.0	4.8 (0.0006)	35.2 ± 0.3	34.9 ± 0.7	0.9 (NS)
Length C (mm)	43.2 ± 4.1	39.2 ± 1.8	10 (0.02)	41.9 ± 2.2	41.7 ± 2.0	0.4 (NS)
PAmid (mm^2)	13.9 ± 1.7	10.6 ± 1.2	31 (<10-4)	11.3 ± 1.3	12.3 ± 1.5	-8.1 (NS)
EAmid (mm^2)	3.7 ± 0.4	2.5 ± 0.5	48 (<10-4)	2.6 ± 0.4	2.9 ± 0.6	-10 (NS)
CortCSA (mm^2)	10.2 ± 1.4	8.1 ± 1.0	26 (0.001)	8.7 ± 1.1	9.4 ± 1.1	-7.4 (NS)
TotvBMC (mg/cm^3)	1324 ± 16	1258 ± 32	5.2 (<10-4)	1266 ± 14	1281 ± 26	-1.2 (NS)
CortvBMC (mg/cm^3)	1858 ± 26	1802 ± 26	3.1 (0.0002)	1794 ± 24	1815 ± 26	-1.2 (NS)

Phenotypes are uncorrected and presented as mean ± sd.

The difference between (GK –F344 and GKxF344F1-F344xGKxF1) are indicated, and nominal *p*-values determined by ANOVA are given.

Table 3A. Bone size related QTLs from male F2 rats.

Chr	Position[a] (cM)	Phenotype	LOD scores[b] Male		
		Phenotype	All N=108	Cross 1 N=66	Cross 2 N=42
1	D1Rat232-D1Rat4 (3-11)	CAfib	2.9	1.2	2.0
1	D1Rat14-D1Rat20 (26-45)	Length S	2.5	2.3	2.6
1	D1Rat20-D1Wox16 (54-67)	EAmid	2.6	3.4	0.7
1	D1Rat136-D1Wox16 (32-67)	CAmid	2.4	2.7	0.6
1	D1Rat20-D1Mgh1 (41-74)	PAfib	3.6	3.3	0.9
2	D2Mit14-D2Arb24 (99-110)	PAfib	0.8	3.6	1.2
2	D2Mit12-D2Arb24 (89-110)	CAfib	0.3	2.7	1.5
3	D3Mit8-D3Mit7 (58-68)	Length S	2.3	3.1	1.3
3	D3Mit8-D3Mit7 (57-69)	Length C	0.6	2.5	0.6
5	D5Rat119 (0-12)	CortBV/TotBV	0.9	3.0	0.4
5	D5Mit10-D5Rat60 (51-68)	CortBV/TotBV	1.1	0.07	2.9
6	D6Mgh11-D3Mit19 (33-55)	CAmid	3.0	1.5	2.2
6	D6Mgh11-D3Mit19 (25-56)	PAfib	2.7	2.3	1.3
6	D6Mgh11- D3Mit19 (25-38)	CortBV	1.7	1.0	3.5
8	D8Mit2-D8Mgh4 (44-64)	Length_S	2.6	1.3	2.0
8	D8Mit5-D8Mit2 (33-55)	PAmid	2.9	1.1	2.4
8	D8Mit5-D8Mit2 (34-56)	CAmid	3.3	1.2	3.2
8	D8Mit5-D8Mgh4 (34-63)	PAfib	2.9	1.3	3.4
8	D8Mit3-D8Mgh4 (39-61)	CAfib	2.8	1.4	2.8
8	D8Mit2-D8Mgh4 (44-63)	TotBV	3.0	0.9	2.7
10	D10Mgh5-D10Mgh4 (74-83)	EAmid	3.4	1.4	2.3
10	D10Mgh5-D10Mgh4 (74-85)	CAmid	2.5	1.9	0.6
10	D10Mgh5-D10Mgh4 (76-85)	PAfib	2.6	2.8	0.8
10	D10Mgh5-D10Mgh4 (73-83)	EAfib	2.6	2.6	1.5
10	D10Mgh5-D10Mit11 (74-91)	CortBV	2.7	1.8	1.2
12	D12Rat15-D12Rat22 (36-53)	TotBV	2.7	1.3	2.2
14	D14Mit10-D14Rat22 (63-82)	EAmid	2.5	1.3	1.0
16	D16Mit5 (0-6)	CortvBMC	2.4	0.8	2.2
17	D17Rat12-D17Mit4 (43-80)	CortvBMC	3.2	3.4	0.2
17	D17Rat12-D17Mit4 (46-79)	TotvBMC	2.8	3.6	0.2
18	D18Mit17-D18Rat11 (29-40)	Length C	1.9	0.07	3.2
20	D20Rat55-D20Rat29 (40-45)	EAmid	0.8	3.1	0.6

Table 3B. Bone size related QTLs from female F2 rats.

Chr	Position[a] (cM)	Phenotype	LOD scores[b] Female		
			All N=98	Cross 1 N=48	Cross 2 N=50
1	D1Mgh2-D1Rat20 (26-51)	Length S	2.5	1.7	1.5
1	D1Rat7-D1Rat20 (17-49)	CortBV	2.9	1.6	2.8
1	D1Wox16-D1Mgh1 (65-81)	CortBV/TotBV	2.7	2.7	0.8
1	D1Mgh34-D1Wox20 (106-125)	EAmid	2.8	0.6	3.0
2	D2Mit14-D2Mit15 (100-125)	Length S	3.1	1.5	1.7
2	D10Rat23-D2Mgh22 (110-134)	CortBV/TotBV	2.6	1.9	0.8
3	D3Mit10-D3Rat46 (0-19)	CAfib	2.4	2.9	1.1
3	D3Rat189-D3Mit8 (27-41)	CortBV	2.4	2.7	1.1
3	D3Rat189-D3Mit8 (24-38)	TotBV	2.0	2.9	0.5
4	D4Rat12-D4Rat37 (29-58)	CAmid	2.5	2.7	1.1
7	D7Mit23-D7Mit7 (17-38)	EAfib	2.8	1.8	1.2
7	D7Rat106-D7Mit5 (44-63)	Length C	2.4	1.3	1.0
8	D8Mit9-D8Mit2 (22-51)	EAmid	2.7	3.6	1.1
10	D13Mit11-D10Rat2 (85-103)	PAmid	2.7	1.5	3.7
10	D10Mgh13-D10Rat2 (90-103)	EAmid	2.4	0.8	3.1
10	D13Mit11-D10Rat2 (82-103)	PAfib	2.5	1.7	2.6
14	D14Mit17-D14Mit10 (45-65)	PAmid	2.8	2.9	0.8
14	D14Mit17-D14Mit10 (40-67)	EAmid	2.4	3.7	0.6

[a] The approximate size of the locus was defined as the region covered by a 1-LOD reduction for any of the bone traits.

[b] Genome-wide suggestive QTLs (LOD≥2.3) are reported.

Cross 1 represents females with GKmtDNA and cross 2 represents females with F344mtDNA.

TotBV = total bone volume; CortBV = cortical bone volume; CortBV/TotBV = cortical bone volume fraction; Length S = straight length; Length C = curved length; PAmid = periosteal area at midshaft; EAmid = endosteal area at midshaft; CAmid = cortical area at midshaft; PAfib = periosteal area at fibula-site; EAfib = endosteal area at fibula-site; CAfib = cortical area at fibula-site; Tot BMC = total bone mineral content; CortBMC = cortical bone mineral content