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HDL cholesterol and bone mineral density: Is there a genetic link?

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Abstract

Overwhelming evidence has linked cardiovascular disease and osteoporosis, but the shared root cause of these two diseases of the elderly remains unknown. Low levels of high-density lipoprotein cholesterol (HDL) and bone mineral density (BMD) are risk factors for cardiovascular disease and osteoporosis respectively. A number of correlation studies have attempted to determine if there is a relationship between serum HDL and BMD but these studies are confounded by a number of variables including age, diet, genetic background, gender and hormonal status. Collectively, these data suggest that there is a relationship between these two phenotypes, but that the nature of this relationship is context specific. Studies in mice plainly demonstrate that genetic loci for BMD and HDL co-map and transgenic mouse models have been used to show that a single gene can affect both serum HDL and BMD. Work completed to date has demonstrated that HDL can interact directly with both osteoblasts and osteoclasts, but no direct evidence links bone back to the regulation of HDL levels. Understanding the genetic relationship between BMD and HDL has huge implications for understanding the clinical relationship between CVD and osteoporosis and for the development of safe treatment options for both diseases.

Keywords

High density lipoprotein cholesterol; bone mass; genetics; pleiotropy

1.1 INTRODUCTION

It has long been understood that cardiovascular disease (CVD) and osteoporosis may be linked [1, 2]. As the American population ages, the incidence of both of these diseases is expected to increase [3, 4]. Lifestyle factors such as smoking, lack of exercise, and eating a high fat diet all increase risk for both conditions [5, 6]. Diagnosis of CVD is associated with increased risk of hip fracture [7, 8] and similarly, studies have suggested that low bone mass in women may be an independent predictor of CVD [9]. Women diagnosed with osteoporosis are at approximately a four fold increased risk of suffering a cardiovascular event and this risk is independent of other CVD risk factors. This risk of a cardiovascular event increases with osteoporosis severity, but appears to be independent of the frailty associated morbidities seen with severe osteoporosis [10]. The common root cause of these two diseases is not completely understood.

Serum lipids have long been known to be associated with risk for CVD. Specifically, low levels of high density lipoprotein – cholesterol (HDL) are associated with increased risk of negative cardiac events [11]. While HDL has many functions that are collectively anti-

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atherogenic, the best-known function of HDL is its role in reverse cholesterol transport. In reverse cholesterol transport, HDL removes cholesterol from the peripheral tissues and transports it to liver. In addition, certain subclasses of HDL have been described as having anti-oxidant and anti-inflammatory properties, both of which likely contribute to the anti-atherogenic effects of HDL [12].

It has been hypothesized that there is relationship between serum HDL levels and bone mineral density (BMD). The aim of this review is to more closely examine the existing evidence supporting or contradicting the hypothesis that these two factors are correlated and/or genetically co-regulated. First, a summary of epidemiological studies investigating the relationship between serum HDL and BMD is presented. A discussion of the covariates that may be influencing the relationship between these two phenotypes and a summary of the data demonstrating the direct interaction between HDL and osteoblasts and osteoclasts follows. Lastly, genetic loci associated with both serum HDL and BMD have been extensively mapped in mouse models and the high degree of co-mapping of loci for these two phenotypes in mice is demonstrated.

2.1 ASSOCIATION BETWEEN HDL AND BONE MINERAL DENSITY

A number of studies have tested for correlation between serum HDL and BMD in human subjects [13–31], as are summarized in Table 1. There is no single consensus that can be reached after reviewing these studies regarding the correlative relationship between HDL and BMD. For a variety reasons, these studies are difficult to compare to one another. First, there are fundamental differences in the compositions of the study cohorts with regards to ethnicity and race of the subjects. It is well understood that BMD is different among various races, with African American's having higher BMD than other racial groups. Ethnicity, a broader term that collectively refers to environmental factors such as cultural practices, diet, activity levels, sunlight exposure etc, as well as genetic factors associated with race may actually have a larger effect on bone than genetics alone [32]. Similarly, HDL is affected by both racial and ethinic differences [33]. The studies presented in Table 1 reflect a larger number of racially and ethnically different populations. A large study of Swedish women (6886 subjects) found a negative correlation between HDL and BMD, but two large studies of Korean women suggest that the correlation is positive, at least in post-menopausal women and three additional large studies found no correlation at all. Thus, ethnic differences may in part account for the lack of consensus findings among these studies. Second, there are significant differences in analysis methods used in the studies in Table 1 as there is no consistent inclusion of covariates in the models. This is discussed further below. Third, many of the studies listed in Table 1 are very small and likely suffer from a lack of power. This raises questions about the validity and interpretability of the findings from these smaller studies. For example, 5 studies have examined HDL and BMD in Korean subjects [13–16, 26]. Two studies, both of which contained over 1000 subjects found a positive relationship between HDL and BMD in post-menopausal women [13, 14]. However no relationship or only a weak negative relationship was found in post-menopausal women in the three much smaller studies [15, 16, 26].

BMD and HDL are affected by factors such as age, gender and menopausal status. The vast majority of the subjects included in the studies done to date were over the age 45. None of the studies reported have examined the relationship between these two factors in cohorts of young healthy adults and no studies have looked at longitudinal changes in BMD and HDL. The three largest studies suggest that there is no association between BMD and HDL in men [25, 27, 29], however, the relationship in women is less clear as several studies have suggested that estrogen status affects this relationship. In sum, there does appear to be an

association between HDL and BMD, but this relationship is strongly context specific and the existing data is insufficient to determine the specifics of this relationship.

2.2 ESTROGEN, HDL AND BMD

It is well understood that incidence of CVD increases dramatically in women after menopause [34]. Furthermore, BMD begins to fall and bone resorption rates increase in women during the peri-menopausal period [35]. Both the rise in CVD and the decline in BMD at menopause are thought, at least in part, to be due to a loss of estrogen. Estrogen has direct physiological effects on both serum lipid metabolism and on the regulation of bone resorption (reviewed in [34, 36]). Hormone replacement therapy (HRT) is associated with an increase in both HDL and BMD in post-menopausal women [34, 37]. Thus, differences in estrogen status could alter the relationship between BMD and HDL in women.

Of the studies presented in Table 1, six studies included both pre- and post-menopausal women. In two studies, both conducted in Korea, a positive association between HDL and BMD was noted in post-menopausal women, whereas no association between these two factors was observed in pre-menopausal women [13, 14]. In contrast, Cui et al, found no association between HDL and BMD, regardless of menopausal status in a very small study, also of Korean women [26]. Wu et al. found a negative correlation between HDL when considering their entire study cohort consisting of Taiwanese men and women, but this significant association was lost when the study cohort was subdivide based on sex and menopausal status [29]. Hsu et al, found no association between HDL and bone mineral content (BMC) in either pre- or post-menopausal women from China [25], but it must be noted that BMD and BMC, while related, are different phenotypes. Areal BMD, the density measured most commonly in human subjects, is the amount of mineral present in bone corrected for by the projected area or size of bone. Bone mineral content is simply the amount of mineral present in the region of interest. While Hsu *et al*. did account for subject height in their analysis, skeletal size was not accounted for [25], making it difficult to compare these findings with the studies in which BMD was measured.

Three studies specifically took use of HRT into account in their analysis. In the study by Makovey et al, a negative association between HDL and BMD was noted in both post-menopausal women taking HRT and in pre-menopausal women, whereas no association was found in the post-menopausal women not on HRT [21]. In the study by Lidfelt *et al.*, a negative association between HDL and BMD was noted in pre-menopausal women as well as post-menopausal women, regardless of HRT use [31]. Lastly, in the large study by Solomon *et al*., after correction for hormonal status, no association was found between serum HDL and BMD [27]. The two large studies of Korean women suggest that in menopausal status affects the relationship between BMD and HDL, at least in certain ethnic backgrounds, however it cannot be determined at this time if it is estrogen per se affects this association.

2.3 GENE BY ENVIRONMENT INTERACTIONS

It has been estimated that up to 85% of the variance in BMD can be explained by heritable factors and the heritability estimates for serum HDL levels range from between 40 and 60% [38, 39]. Genetic differences in the study cohorts are a likely source of the lack of concordance among the studies examining the relationship between HDL and BMD [27]. Furthermore, studies have demonstrated gene-environment interactions for both BMD [40–42] and HDL levels [43–46]. Some key variables which have been implicated in gene-environment interactions, such as smoking, were not accounted for in some of the smaller studies listed in Table 1, but were used in other studies, which may also explain the lack of consensus among these studies [24, 46]. Lastly, some of the studies listed in Table 1 are

small, and are therefore too underpowered to account for the combination of genetic and environmental influences on this relationship.

2.4 THE USE OF RODENT MODELS TO BETTER UNDSTAND THE CORRELATION BETWEEN BMD AND HDL

A number of methods have been developed to measure serum and plasma levels of HDL. The gold standard is to use the ultracentrifugation method. This method is very labour intensive and requires large sample sizes, making this method undesirable for use in large high-throughput studies or in studies where there is a limited sample volume. A number of direct methods for measuring HDL have been developed, but not all of these techniques can be used with mouse samples. For example, a number of studies have now suggested that the PEGME method yields unreliable results in samples taken from hyperlipidemic mice [47] whereas HDL measured by the Polymedco/Denka method shows good correlation with measurements made by ultracentrifugation [48]. The Denka method has been reliably used by a variety of investigators to identify genetic loci associated with this phenotype in mice [48].

In studies using animal models, environmental variables such as diet can be can be controlled for, and in rodent models, genetic background can be held relatively constant. A large number of strain surveys examining serum lipids and BMD have been conducted in mice and these data can be used to more closely examine the effect of genetic background on the response of HDL and BMD to age and diet. Data for three genetically distinct strains of mice is presented here to highlight the importance of considering age, diet and genetic background. Age does not affect either serum HDL levels or BMD in C57BL/6J mice (Fig 1A and B), the most commonly used strain of laboratory mice (The mouse phenome database project data sets: Yuan3 and Ackert1, [49],). Both serum HDL levels ($P>0.001$) and BMD ($P=0.0017$) decrease with age in BTBR *T+ Tf*/J mice when comparing 6 month old mice to 18 month old mice (Fig 1A and B). However, when comparing 6 month old mice to 12 month old mice of this strain, a significant decrease is observed in HDL ($P = 0.003$) whereas no difference in BMD is observed ($P=0.626$), demonstrating that the rate and pattern of age associated changes in these two phenotypes is important to consider. In 129S1/SvImJ mice, both serum HDL and BMD increase with age ($P>0.001$, Fig 1A and B). Similarly in humans, different ethic populations show different patterns in age related changes in HDL [50–53] further supporting the hypothesis that genetic background affect age related changes in HDL. In a separate strain survey, the impact of dietary fat on serum HDL was examined in a variety of inbred strains (The mouse phenome database project data set: Paigen 2, [49]). Serum HDL increases dramatically in 129S1/SvImJ mice fed a high fat diet ($P<0.001$) whereas there was no change in this phenotype in either BTBR *T+ Tf*/J ($P=0.624$) or C57BL/6J ($P=0.47$, Fig 1C). While data is not available with regards to the impact of dietary fat intake on BMD in all three of these strains, studies have demonstrated strain specific responses to dietary fat intake with regards to BMD [40]. In summary, in these three strains of mice, three completely different patterns of genetic background by diet and genetic background by age interactions are obvious for HDL. Furthermore, the timing of age related changes in HDL may or may not coincide with age related changes in BMD.

Both serum HDL and BMD are heritable complex traits, meaning that multiple genes regulate these two phenotypes. A large number of genetic loci have been mapped for both of these phenotypes in mice in a number of independent studies. A more complete description of these genetic mapping efforts is discussed below. The Yuan3 and Ackert1 phenotype data sets contain HDL and BMD phenotype data for many of the strains used to map the genetic loci associated with these two phenotypes. For both of these data sets, the mice were fed the same low fat diet and were raised in the same animal facility. Each mouse of a particular inbred strain can be essentially considered an identical twin of every other mouse of that

strain, but each strain of mice is, to a certain degree, genetically distinct from other inbred strains [54]. Thus, there is minimal variation in measures of phenotypes that are largely genetically regulated. By correlating the strain average BMD and HDL data, we can determine if genetic background affects the relationship between these two phenotypes. As is presented in Figure 1D and 1F, at 6 months of age, there is a positive correlation between HDL and BMD when examining the inbred strains used to map genetic loci for these two phenotypes. Similarly, a positive, albeit weaker, correlation between HDL and BMD is observed in when comparing the 12 month old mice of the same strains (Figure 1E and 1F). This suggests that strains of mice trending towards high BMD tend to have higher serum HDL levels. An example strain would be MRL/MpJ (MRL). At 12 months of age, female MRL mice have the highest BMD of the strains examined in the Ackert1 data set and have one of the highest HDL measures in the Yuan3 dataset. Given that lower BMD and lower HDL levels are both associated with increased risk for osteoporotic fracture and CVD respectively, this positive correlation is not unexpected [4, 10, 38]. However, as both of these phenotypes are polygenetic, there will be strains of mice that do not follow the pattern of high BMD being associated with high HDL because of the collection of alleles inherited for both phenotypes. For example, at 6 months of age PWD/PhJ female have high serum HDL, but do not have high BMD. Strain survey data, such as these data sets, are useful for choosing strains for the study of single phenotypes as well as for the study of putatively co-regulated phenotypes. The problem with strain surveys is that there are a limited number of possible allelic combinations available for study. Future studies need to be undertaken in which the relationship between BMD and HDL is examined in genetically diverse mouse strains, such as the collaborative cross [55].

3.1 INTERACTION BETWEEN HDL AND OSTEOBLASTS AND OSTEOCLASTS

Increasingly, it has been appreciated that HDL can act directly on osteoblasts and osteoclasts in bone. Studies using osteoblast-like cells lines have suggest that these cells are able to internalize and degrade certain subclasses of HDL particles. In addition, these cells express scavenger receptor class B type I (SR-B1), scavenger receptor class B type II (SR-BII) and CD36 cell surface receptors, which are involved in the selective uptake of cholesterol esters from HDL in other cell types. It has been demonstrated that osteoblasts are indeed capable of selective uptake of cholesterol esters from HDL, but is unclear if this uptake is mediated by the SR-BI, SR-BII or CD36 receptors [56]. In atherosclerosis, hyperlipidemia is associated with an accumulation of LDL, and the subsequent oxidation of this LDL in the subendothelial matrix of arterial walls. Some studies have suggested that HDL can inhibit this lipid oxidation, but the mechanism is not completely clear (reviewed in [12]). Brodeur and collegues have shown that oxidized LDL can induce apoptosis of osteoblasts and that this effect can be abrogated by the addition of HDL [57]. In osteoclasts, the removal of cholesterol from these cells by HDL particles can induce apoptosis whereas the delivery of cholesterol via low-density lipoprotein cholesterol (LDL) increases osteoclast survival [58]. *Scarb1*, the gene that codes for both SR-BI and SR-BII, appears to also be expressed in the osteoclast (biogps.gnf.org).

The HDL particle is composed of a variety of proteins, fat-soluble vitamins and steroid esters [59]; many of the components of HDL have been shown to directly affect bone metabolism. Specifically, fatty-acid esters of estrogen are known to be a component of HDL [60]. These steroid esters can be taken up by cells from HDL via SR-BI receptors and converted to free steroids [60, 61]. It is well appreciated that estrogen has direct and important functions in basic bone biology and that decreased levels of estrogen with age are a key component of age related loss of bone mass [36]. It is unclear if HDL associated estrogen esters are involved in bone metabolism. Fat soluble vitamins such as Vitamin E

(alpha-tocopherol and delta-tocopherol) [59] and vitamin K (phylloquinone and menaquinone) [62] are also key components of HDL. Application of Vitamin E to calvarial osteoblasts in cultures results in an inhibition of early stage osteoblast maturation [63]. While the epidemiological data gathered thus far has not resolved the relationship between serum levels of Vitamin K and bone mass, in vitro studies have suggested that Vitamin K increases mineralization by the osteoblast and decreases expression by the osteoblast of the pro-osteoclast maturation factor, RANKL [64]. There is insufficient data at this to time to determine if HDL particles regulate bone formation and resorption by the delivery of physiologically relevant compounds to the bone micro-environment.

4.1 PLEIOTROPY

Pleiotropy, by definition, means that one gene affects multiple phenotypes. By extension, it can be assumed that a mutation or polymorphism in a gene with pleiotropic function would be associated with the coincident change in two or more phenotypes [65]. The pleiotropic effect of a gene could be either via direct action on two or more phenotypes, which is true pleiotropy, or indirect in action [66]. In the case of HDL and BMD, a truly pleiotropic gene would in some way directly alter the levels of HDL in the serum by changing production or clearance of HDL and simultaneously affect bone mass. Thus, for a true pleiotropic gene, it can be assumed that HDL particles themselves do not alter the formation or resorption of bone and no hormones or other factors secreted from or released from the bone in any way regulate HDL levels. This seems highly unlikely given that HDL is known to interact directly with both osteoblasts and osteoclasts, as was described in the previous section. An example of indirect model would be one in which some metabolic factor is carried to the bone directly by HDL and taken up by an osteoblast, which causes a change in osteoblast function. In this model, a polymorphism in a gene that regulates serum HDL levels would be observed to change bone mass, but the bone mass is only changed because the levels of HDL are changed. This model appears more plausible based on the available data.

3.2 EVIDENCE FOR SHARED GENETIC REGUALTION OF HDL AND BMD

As has already been described, both HDL and BMD are highly heritable traits. A number of genes have been examined for both HDL and BMD independently. In Table 2, several genes are presented for which a genetic association between serum HDL has been reported in the literature and in separate studies, a role in basic bone biology and or a genetic association with BMD has been reported. This list is not a comprehensive listing of gene associated with BMD and HDL but rather is a listing of genes for which there is multiple lines of evidence supporting the hypothesis that the gene regulates both phenotypes. It is unclear at this time how many, if indeed any, of these genes are truly pleiotropic.

The mouse genome is 95% identical to the human genome, making mice an excellent animal model for the study of human diseases [67, 68]. A quantitative trait locus (QTL) is a region of the genome that is associated with a given quantitative trait such that a gene or genes located at that locus regulate the trait of interest [69]. QTL mapping in mice has been a successful method for determining the mechanisms of genetic regulation for a variety of complex traits. Both BMD and HDL are polygenic traits and a large number of QTLs have been mapped for both phenotypes [70–78]. In Figure 2, the known QTL for these two phenotypes are presented. It can quickly be seen in this figure that there are a number of regions wherein the peak location for QTLs for these two phenotypes co-map. Some of the BMD and HDL QTLs were mapped in the same set of mice. Where the peak location of QTLs for BMD and HDL map very near to each other, it cannot be determined, with the existing data, if the QTLs are co-mapping/ linked QTLs (i.e. QTLs for which the underlying genes map to locations very near to each other) or if these QTLs are indeed caused by pleiotropic genes. It is highly unlikely that these QTLs are mapping together purely by

chance. It is much more likely that there are at least some loci wherein the underlying gene co-regulates both phenotypes either directly or indirectly. Preliminary studies suggested that *Apoe* may be just such a gene. The *Apoe*$^{-/-}$ mice have increased BMD and increased bone formation, but have decreased serum HDL levels [79, 80]. However, studies in humans have shown that, while polymorphism in *APOE* are associated with changes in serum lipids, they are not similarly associated with changes in BMD when the two phenotypes were examined in the same cohort. The authors concluded from their data that the association between serum lipids and osteoporosis was indirect in nature [24]. It is also apparent from this figure that there are loci that are private to HDL and loci private to BMD. This could explain why strains like PWD/PhJ do not follow high BMD equals high HDL relationship seen in other strains as, in any given inbred strain of mice, the final the phenotype observed for any complex polygenic trait will reflect the sum of all of the alleles inherited for that trait. The existence of private loci does not negate the possibility that there is a genetic relationship between HDL and BMD, but rather emphasizes that both BMD and HDL are regulated by multiple mechanisms and that only some of these mechanisms are shared between BMD and HDL.

5.1 CONCLUSIONS AND FUTURE DIRECTIONS

As summarized, a number of correlation studies have attempted to determine if there is a relationship between serum HDL and BMD. The human studies serve to emphasize that this relationship is confounded by a number of variables including age, diet, genetic background, gender and hormonal status. Collectively, this data suggests that there is a relationship between these two phenotypes, but that the nature of this relationship is context specific. Most of the study cohorts examined to date consisted of postmenopausal women. We have very little knowledge about the relationship between HDL and BMD in younger, healthy adults. Furthermore, we have insufficient data about the importance of co-morbidities and environmental factors such as diet in human and how they influence the relationship between BMD and HDL. While it is temping to suggest that bigger studies or studies of selected cohorts are needed, the specifics with regards to who to study and what to consider in the analysis models remain unclear.

Studies in mice, in which environment, genetics and age can be controlled, are proving to be very helpful to identify how HDL and BMD are related. The examination of inbred strains of mice clearly demonstrates that dietary fat affects HDL in a strain specific manner. Using mice to determine which co-factors should be included in a candidate gene association study has previously been successfully used to find genes associated with BMD [40] and animal models will be very helpful to identify the environmental factors that influence the relationship between BMD and HDL. Studies in mice plainly demonstrate that genetic loci for BMD and HDL co-map and transgenic mouse models have been used to show that a single gene can affect both serum HDL and BMD. A number of centers worldwide are currently involved in broadly phenotyping the mice generated by International Knockout Mouse Consortium and plans are underway to establish additional phenotyping centers. Already, exciting unknown gene functions and novel disease models have been identified as part of this endeavor. Undoubtedly, this effort will identify new genes associated with both bone mass and with serum lipids, which will increase our understanding of the mechanisms by which these two phenotypes are associated. Numerous candidate genes for HDL and BMD have been identified in genome wide association studies (GWAS) of human subjects [39, 70, 81, 82]. While there is a well-recognized need to study pleiotropy at the genome wide association level, the statistical methods and analysis tools for this type of work are still in development [83, 84].

In sum, there is sufficient evidence to conclude that BMD and HDL are genetically linked, but additional studies in human subjects will be complicated due to the large number of factors that affect this relationship and new tools will have to be developed for these studies. Work completed to date has demonstrated that HDL can interact directly with both osteoblasts and osteoclasts, but no direct evidence links bone back to the regulation of HDL levels. Understanding the genetic relationship between BMD and HDL has huge implications for understanding the clinical relationship between CVD and osteoporosis and for the development of safe treatment options for both diseases.

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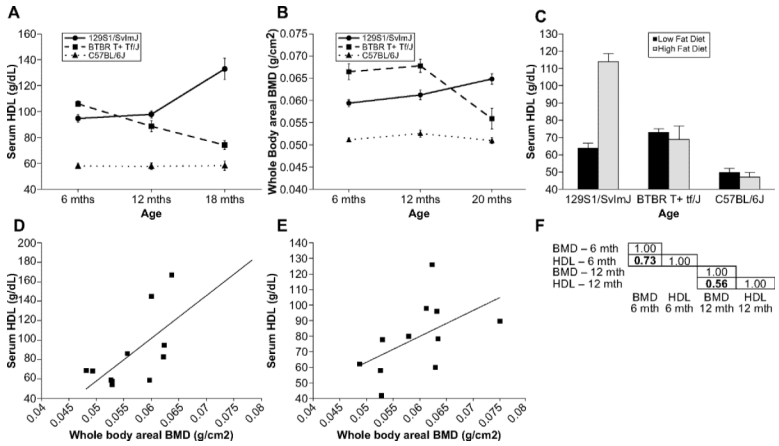

Figure 1. Serum HDL and bone mineral density (BMD) in inbred strains of mice

Data for a variety of phenotypes in inbred strains of mice can be found in the Mouse Phenome Database [49]. **A**. Serum HDL data for female C57BL/6J, BTBR *T+ Tf*/J and 129S1/SvImJ mice was accessed from Project Yuan3. In this longitudinal study, mice were fed a standard low fat diet and serum HDL was measured at 6, 12 and 18 months of age using the Polydmedco/Denka Seiken methodology. **B**. Whole body areal BMD (aBMD) data from female C57BL/6J, BTBR *T+ Tf*/J and 129S1/SvImJ mice was accessed from Project Ackert1. In this cross-sectional study, mice were fed a standard low fat diet and aBMD was measured by dual X-ray absorptiometry (DXA) at 6, 12 and 20 months of age. The data in the Yuan3 and Ackert1 projects were collected by the Jackson Aging Centre and the mice were housed under similar environmental conditions. **C**. Serum HDL data for female C57BL/6J, BTBR *T+ Tf*/J and 129S1/SvImJ mice was accessed from Project Paigen2. In this study, mice were fed a standard rodent chow up to the age at which baseline HDL was measured (between 7 and 10 weeks of age). The mice were then placed on a high-fat, high-cholesterol atherogenic diet (pga.jax.org/athdiet.html) for 17 weeks and HDL was measured again in the same mice. **D**. A scatterplot plot comparing HDL versus BMD is presented using strain average data for the female mice from the 6 month old age cohort from the Yuan3 and Ackert1 datasets. Only those strains in which genetic loci for BMD and or HDL had been mapped are presented. **E.** A scatterplot plot comparing HDL versus BMD is presented for the same strains as in D, but using data from the 12 month old cohort. **F**. The correlation between the BMD and HDL data from the Yuan3 and Ackert1 datasets for the 6 and 12 months cohorts. Only strains used in genetic mapping studies were used in the correlations.

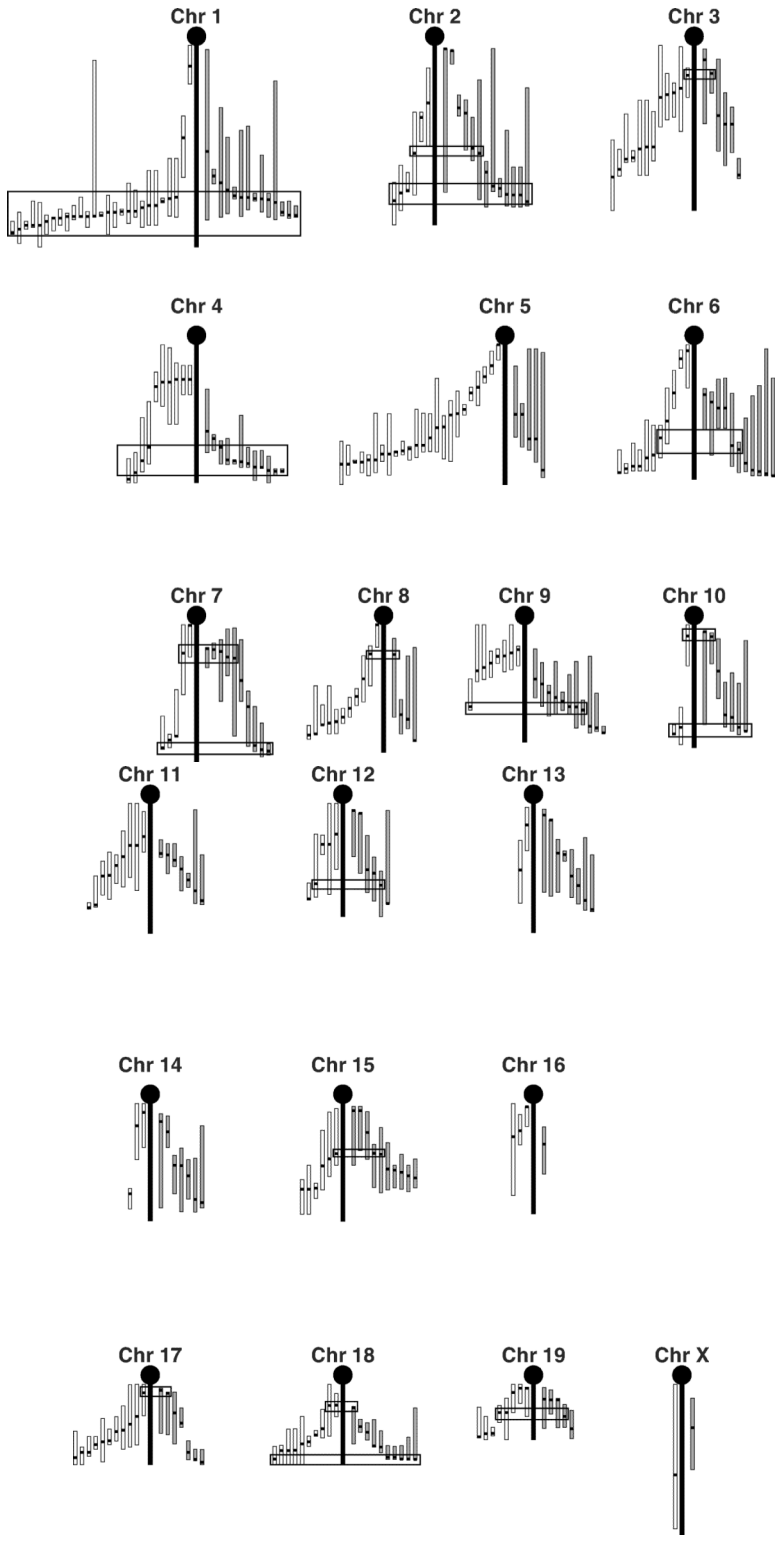

Figure 2. Quantitative trait loci (QTL) for HDL and BMD

The literature was searched for the known HDL and BMD QTL mapped in mice [70–78]. In total 155 BMD QTL and 175 HDL QTL were identified. These QTL are plotted as follows: The central vertical black bar represents the chromosomal backbone. The HDL QTL are

drawn on the left of the chromosome (white bars) and the BMD QTL are drawn on the right (grey bars). For each QTL, the peak location is indicated by a horizontal thick black tick mark and the vertical white or grey bars represent the 95% confidence intervals (CI). The CI are presented to scale using the literature listed CI when available. When no CI was listed, a conservative 20 cM interval was drawn to each side of the peak location, or to the top or bottom of the chromosome if that was closer. Black rectangles highlight locations of concordant peak locations when comparing the HDL and BMD QTL. For chromosomes for which there is little agreement among the crosses with regards to peak location for HDL (i.e. Chromosome 5), no attempt to identify concordant BMD-HDL QTL peaks was made.

Table 1

Relationship between serum HDL-C and BMD.

Study cohort	Relationship between HDL-C and BMD	Ref.
1,234 pre- and 931 post-menopausal Korean women	Positive association between HDL-C and lumbar spine BMD in post-menopaual women.	[13]
4,613 pre- and 2,661 post-menopausal Korean women	Positive association between HDL-C and lumbar spine BMD in post-menopaual women.	[14]
295 Korean men and 166 woman	Positive association between HDL-C and BMD in men	[15]
399 post-menopausal Korean women	Weak negative association between HDL-C and lumbar spine and femoral neck BMD	[16]
289 Caucasian Spanish men (64±9 years of age)	No association between HDL-C and lumbar spine and femoral neck BMD Half of subjects had hypercholesterolemia	[17]
620 men and 635 post-menopausal Dutch women (65–88 years of age)	Both men and women in the highest quartile of HDL-C had significantly lower BMD of the calcaneus compared to the lowest quartile (as measured by quantitative ultrasound)	[18]
107 post-menopausal Turkish women	No difference in serum HDL-C levels in osteoporotic versus non-osteoporotic patients	[19]
224 pre-, 273 post-menopausal Australian women	Negative association between HDL-C and total hip, femoral neck and whole body BMD in pre-menopausal and post-menopausal women on HRT.	[21]
101 osteoporotic and 72 normal post-menopausal Italian women	HDL was significantly higher in osteoporotic women compared to non-osteoporotic controls.	[22]
465 men and 448 women from the United Kingdom	Negative association between HDL-C lumbar spine BMD and total femoral BMD in women and between HDL-C total femoral BMD in men.	[23]
1176 post-menopausal Danish women	No association between HDL-C and BMD of either the lumbar spine or total hip	[24]
7137 men, 4585 pre- and 2248 post-menopausal women from China	No association between HDL-C and whole body BMC in any group	[25]
375 pre- and 355 post-menopausal Korean women	No association between HDL-C and BMD at lumbar spine or hip	[26]
13,592 American men and women	Positive relationship between HDL and BMD in crude analysis. No association between HDL-C and BMD after adjustment for age, sex and menopausal status	[27]
717 women and 265 men from Italy	Negative associations with HDL and whole body BMD in women (not significant after correction for %Fat, lean mass and age). Negative association between HDL and hip BMD in men, even after correction	[28]
2830 women and 2170 men from Taiwan	Negative association between HDL-C and BMD of the wrist in whole cohort. No association when cohort is divided by sex and menopausal status.	[29]
214 post-menopausal Japanese women	Positive association between HDL-C and BMD of the lumbar spine and radius	[30]
6886 Swedish women	Negative association between HDL-C and BMD of the wrist regardless of hormonal status.	[31]

Table 2

Genes associated with both HDL and BMD.

Gene symbol	Gene name	Evidence of genetic association	Ref.
ABCG8	ATP-binding cassette, subfamily G (WHITE), member 8	Was identified as a candidate gene for a mouse HDL QTL on Chromosome 17. Polymorphisms are associated with serum HDL levels in human subjects. *Abcg8*$^{-/-}$ mice have higher BMD.	[85–88]
APOE	apolipoprotein E	*Apoe*$^{-/-}$ mice have increased BMD. Well-established genetic association with HDL in humans. Inconsistent association between APOE polymorphisms and BMD and fracture in humans.	[38,79,89–93]
ESR1	estrogen receptor 1 (alpha)	Is one of two receptors for estrogen. Loss of estrogen at menopause is associated with increased osteoclast activity. *Ers1* knockout male mice exhibit higher BMD and higher serum HDL levels.	[36,94–96]
GHRH	growth hormone releasing hormone	Signals for release of growth hormone. Growth hormone acts both directly and indirectly on bone. Patients with growth hormone deficiency have low levels of HDL and have low bone mass. Polymorphisms in the GHRH gene are associated with proximal femur and spinal BMD.	[97–99]
IL6	interleukin 6	*Il6*$^{-/-}$ mice have decreased BMD. Polymorphisms associated with both HDL and BMD in human subjects.	[100–108]
MTHFR	5,10-methylenetetrahydrofolate reductase	Mutations in this gene causes hyperhomocysteinemia, a risk factor for both CVD and osteoporosis. Polymorphisms in this gene are associated with both HDL and BMD in human subjects.	[109–113]
PON1	paraoxonase 1	Numerous studies have linked polymorphisms in this gene with HDL levels. Polymorphism in this gene may be associated with femoral neck and spine BMD in humans. Other studies have suggested no association with BMD, but association with fracture.	[38,114115]
PPARG	peroxisome proliferator activated receptor gamma	*Pparg*$^{+/-}$ mice have higher bone mass. Polymorphisms in *PPARG* interact with dietary fat to affect BMD in humans. The common *C161T* and *Pro12Ala* polymorphisms are associated with HDL levels in humans.	[40,116–121]
TNF	tumor necrosis factor	*Tnf*$^{-/-}$ mice have increased bone mineral density. Polymorphisms in this gene are associated with both HDL and BMD in human subjects.	[122–127]