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Using mouse genetics to understand human skeletal disease

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ABSTRACT

Technological advances have enabled the study of the human genome in incredible detail with relative ease. However, our ability to interpret the functional significance of the millions of genetic variants present within each individual is limited. As a result, the confident assignment of disease-causing variant calls remains a significant challenge. Here we explore how mouse genetics can help address this deficit in functional genomic understanding. Underpinned by marked genetic correspondence, skeletal biology shows inter-species similarities which provide important opportunities to use data from mouse models to direct research into the genetic basis of skeletal pathophysiology. In this article we outline critical resources that may be used to establish genotype/phenotype relationships in skeletal tissue, identify genes with established skeletal effects and define the transcriptome of critical skeletal cell types. Finally, we outline how these mouse resources might be utilized to progress from a list of human sequence variants toward plausible gene candidates that contribute to skeletal disease.

1. Introduction

Since the publication of the human genome nearly 2 decades ago, a wave of technologies enabling the system-level examination of gene sequence, expression and regulation have been developed [1–3]. These have made it markedly cheaper, quicker and easier to study the molecular landscape in health and disease. Genomic sequencing is one such technology that is commonly used to investigate the genetic basis of disease in the laboratory and increasingly the clinic [4]. However, this data is only as useful as our capacity to interpret it. Currently, our understanding of how this genetic variance contributes to human skeletal health remains limited.

Human genomics allows the identification of genes and loci that have a significant influence on health. Rare gene-mutations that cause monogenic and mendelian diseases can lead to the discovery of new genes that influence human health [5]. The nosology of skeletal genetic disorders catalogues over 350 genes that cause severe skeletal dysplasias in humans, many identifying key molecular pathways that control skeletal function [6]. Population scale genetic screens, such as genome wide association studies (GWAS), enable the association of genetic variants with common, genetically complex, clinically significant traits [7]. One of the largest GWAS published recently identified over 1000 genomic loci significantly associated with of bone mass, a key risk factor and diagnostic metric for osteoporosis explaining ~20% of trait genetic heritability [8]. However, sequencing on a population scale is also confirming another important fact in genomics - most variation does not cause disease. This was exemplified in a recent study of the genomes of 511 healthy individuals over 80 years old, with no chronic diseases and who are not taking medications [9]. This 'wellderly' cohort identified > 100,000 rare (< 1% frequency) genome mutations that affect protein-coding gene sequences, that did not cause overt disease in the people that carry them. This normal variation presents a serious challenge to deciphering which of the millions of variants in an individual are significant in disease, and leads to large lists of putative disease-causing gene-candidates. In these instances, the ability to identify disease causing mutations among the noise of 'normal' genetic variation hinges on our detailed understanding of the regulation and function of the gene involved.

To establish cause and effect relationships between genes and diseases often requires manipulation and experimentation. While this is not possible in humans for ethical reasons, the laboratory mouse, *Mus musculus*, has proven invaluable in the search for functional understanding of genes and genomes. Mice have been used in genetic research for more than a century, stemming from many physiological and genetic similarities with humans, owing to a common mammalian ancestor ~75 million years ago [1,10]. This similarity, combined with practical factors of size, cost, high reproductive output, and the wealth of knowledge stemming from decades of study make the laboratory

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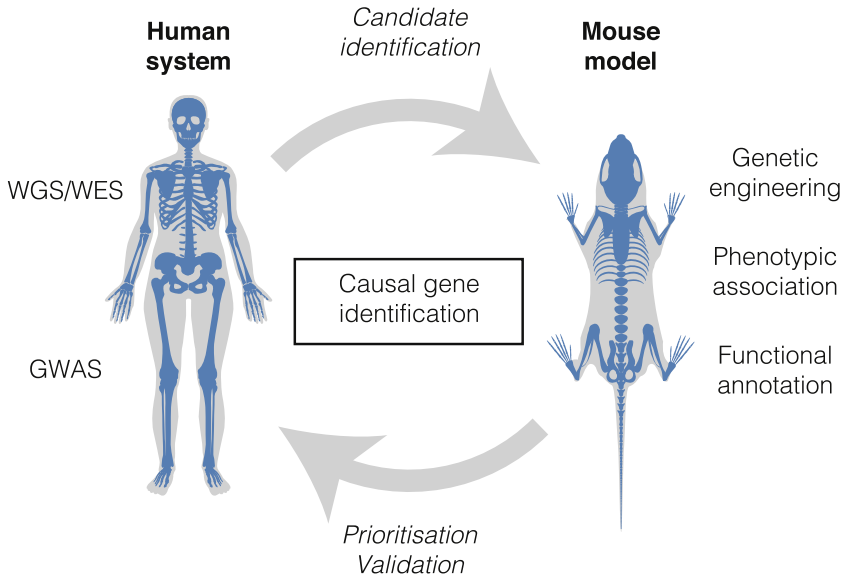

Fig. 1. Using mouse genetics to identify genes causing human skeletal disease. The use of mouse models can facilitate the investigation, experimentation and validation of causal disease genes identified in human whole genome/exome sequencing (WGS/WES) and GWAS data.

mouse the most commonly used animal model used in research [11]. A core strength of using mice as a model of human physiology is the opportunity for genetic manipulation, enabling the direct testing of gene function within the context of an intact *in vivo* mammalian system [12]. Utilising genome engineering technologies to simulate and study gene mutations identified in humans has seen the development of mouse models of a range of human skeletal diseases, from sclerosteosis to osteogenesis imperfecta [13,14]. These models provide insights into the molecular mechanisms of disease, linking genotype to phenotype, and enabling the development and testing of therapeutic strategies.

With rapid advances in genomic sequencing, gene editing technologies, and the increasing scale of international collaborative efforts, the mouse genomics community has developed a number of tools and resources to understand gene function. The aim of this article is to demonstrate how mouse genomics may help guide the search for causal genes in human skeletal disease (Fig. 1). We highlight key resources connecting mouse genotype with skeletal phenotype, examining how their similarities and differences provide unique insights into the genetic architecture that governs bone morphology and function. We examine how curated databases and transcriptomic data may be useful to identify genes important to skeletal biological processes and bone cell function. Lastly, we describe a hypothetical workflow utilising these resources to illustrate how mouse genomics can help address the challenge of refining a list of human gene mutations into plausible disease-causing candidates.

2. The mouse as a model of the human skeletal system

A model of a system is a theoretical description used to aid the understanding of how the system works or may work. Models are used in contexts where it is impractical or impossible to gain a complete understanding of the system by studying the system alone. A model does not have to be a perfect replica of the system to be useful, however a thorough understanding of the specific assumptions and limitations of the model-system relationship is critical to be used effectively. The use of mouse genomics as a model of the human skeletal system hinges on the assumption of similarity of the bone physiology and regulatory genetic architecture between the two species. While there are many lines of evidence which indicate this is a fair assumption, there are some important differences which need to be understood when leveraging mouse genomics to understand human gene function.

While it is obvious mice are not just hairy little humans, there are many similarities between the human and murine skeletal system which make it a valuable model for understanding skeletal biology [15]. The broad patterns of bone development and growth are well conserved between humans and mice. The conversion of a cartilage anlagen to a mature bone by endochondral ossification and the accretion of hydroxyapatite into collagen1-α1 scaffolds is similar between species. While linear growth and the presence of open growth plates continues in mice for a greater period beyond sexual maturity; both display rapid initial growth with pubertal increases, followed by a period of relative stability, but then undergo age-related declines in both cancellous and cortical compartments. The average life span of a mouse is 2–3 years, thus these phases are accelerated, as is the bone remodelling cycle, which takes several months in humans and several weeks in mice. However, the cellular mediators of these processes are the same and osteoblast, osteoclast and osteocyte biology has been effectively explored in parallel between human and mouse.

There are also important differences between the human skeleton and that of the mouse. The lack of osteonal cortical bone remodelling in mice may be the most overt difference between species. While blood vessels do exist on murine cortical bone [16], there is no Haversian system. However, intracortical remodelling does increase cortical porosity in aging mice [17], an important hallmark of age-related bone loss in humans [18]. While the cellular mechanics of Haversian and surface-based remodelling are similar, whether there are differences in the molecular machinery of each process is yet to be defined. Another important distinction is the lack of spontaneous fracture in wild type mice. This may involve underlying biomechanical differences between the species, or responses to limited mobility and controlled housing conditions. Even so, outside of significant pharmacological or genetic intervention, mice do not suffer ‘osteoporotic’ fractures.

Critical for studying therapeutic interventions, the fundamental controllers of bone metabolism are similar between human and mice. Calcaemic pathways involving PTH and vitamin D3, bone lytic pathways involving RANKL and OPG, and bone formation process involving RUNX2 and WNT pathways are conserved between species. Sex hormone regulation of bone mass is consistent. Mice approach the murine equivalent of perimenopause around 9 months and are reproductively senescent by around 12 months, driven by follicular atresia as in humans [19,20]. However, it must be noted that despite these changes with age, mice remain functionally oestrogen sufficient into very old

age: maintaining uterine weight, and suppressing bone remodelling [21]. This notwithstanding, the ovariectomised mouse remains a pivotal model for postmenopausal studies, with caveats of very rapid bone loss and common application prior to extensive age-related bone loss.

Aiding the extrapolation of mouse genomics to human pathophysiology is the concordance between the mouse and humans at the molecular level (comprehensively reviewed in [22]). Shortly after the completion of the human genome, the complete sequence of the mouse genome was reported, and with it, detailed comparisons of the genetic architecture between each species [1].

The mouse genome is ~14% smaller than the human genome yet the number of genes is very similar, with 58,721 and 54,446 genes identified in the most recent GENCODE annotation in the human and mouse genomes, respectively ([23], https://www.gencodegenes.org). Protein-coding genes comprise 33% (19940) of annotated genes in human, while 40% (21969) in mice, with long non-coding RNAs (lncRNAs), small non-coding RNAs and pseudogenes comprising the remainder. While this direct comparison of gene annotations in terms of number of number of gene types should be interpreted cautiously, nearly all human protein-coding genes have an orthologous protein-coding sequence in the mouse genome [1]. This includes ~80% of genes that have a direct 1–1 ortholog mapping, and an overall conservation of gene arrangement of 90% between the two species. Indeed, these statistics indicate a broad similarity between the human and mouse genomes affirming the use of *Mus musculus* as a model of *Homo sapiens*. However, there are important differences between the species which need to be considered when using mice as a model of human genetics. At the level of base-sequence, only ~40% of the human genome sequence can be directly mapped to the mouse genome [1]. Additionally, there are numerous examples where genes from orthologous sequences differ in their functional effects [24,25]. Thus, the conservation of specific genes should be established to ascertain appropriateness of the extrapolation of specific mouse models of human physiology.

Another important measure of the genomic similarity between humans and mice is the allelic variation between individuals. Inbred mouse strains are commonly used in laboratory experiments as their genetic and phenotypic characteristics have been comprehensively studied [26,27]. Inbreeding standardises genetic background, reducing allelic heterozygosity and limiting variability to defined genomic regions for each strain [28]. This lack of genetic variation within inbred mouse lines does not reflect the genetic diversity of outbred human populations [29]. Moreover, numerous studies have demonstrated that the skeleton varies significantly between inbred lines, and that the result of genetic manipulation and other experimental interventions can be strongly mouse-strain dependent [30–35]. Thus, the effect of standardising genetic background may obscure important information regarding the influence of genetic diversity on gene function and variant effects in human populations. To address this, there are a number of initiatives working to establish mouse collections with similar levels of allelic variation to humans through crossing inbred lines and outbreeding [36–38]. These genetically diverse models show a level of phenotypic variance concordant with that of human populations, potentially enabling to the discovery of more robust genotype-phenotype relationships [37]. However, studies that use such models require more mice than those that use a single inbred mouse line, raising ethical and financial concerns [38]. As is often the case, the choice between inbred lines and outbred strains is best guided by the specifics of question being examined. For this reason, the genetic background in terms of strain and allelic variation should be taken into careful consideration when using mice models to understand human genetics.

Beyond DNA sequence, the conservation of gene expression and regulation is another important measure of the molecular similarity between mice and humans [22,39]. Unlike the genome, which is largely the same in every cell type, gene expression and regulation is tissue and cell type specific. Several studies have compared the transcriptome between homologous tissues from mice and human tissues to establish the interspecies fidelity of gene expression. Conflict in early reports comparing gene expression between species using array technology was thought to stem from data handling and normalisation strategies, with later reports indicating broad similarity between homologous tissues [40,41]. However, recent transcriptome sequencing studies indicate conservation of the expression between humans and mice is somewhat tissue, and gene, dependent [42,43]. These findings highlight an important gap in our understanding of the use of mouse genetics as a model of human skeletal physiology: to date, there has been no direct estimate of the human-mouse conservation of molecular expression in skeletal tissues. This is largely owing to a dearth of transcriptome sequencing and gene-regulatory datasets from bone tissues and key skeletal cell types for either humans or mice (explored further in Section 3). Heuristically, the physiological similarities at the morphological, mechanical and cellular level may imply a similarity in molecular processes regulating the human and mouse skeleton [15]. Moreover, the phenotypic concordance of human skeletal dysplasias with mouse models of causal mutations indicates the functional conservation of key genes that influence the skeleton [44]. Together, these measures suggest the assumption of molecular similarity in the skeleton between human and mouse is reasonable. However, a direct comparison of gene expression in skeletal tissue between human and mice is required to ascertain the true value of mice as a model of human skeletal genetics.

3. Connecting genotype with skeletal phenotype

Using mice models to study skeletal biology affords opportunities for genetic manipulation not possible in humans. Gene editing technologies have advanced considerably in recent decades and now enable a broad spectrum of gene modifications [12,45]. These include sequence insertions and deletions, conditional induction and tissue-specific rearrangements involving single nucleotides to large transgene constructs. This toolset allows the engineering of complex experimental mouse models to deduce gene function or mimic genomic variations apparent in patient populations.

Since the inception of these technologies, they have been used to model gene mutations identified in humans and study their effects on phenotype at the morphological, cellular and molecular level [46]. These have most commonly been hypothesis-driven approaches to mouse model generation provide compelling evidence of the identification of causal mutations underlying human disease, and are important to establish disease models for protracted investigation. In addition to these directed approaches, a number of resources have been established which compile comprehensive collections of *in vivo* phenotypic data collected from genetically engineered mouse models, including the skeleton [47]. In contrast to hypothesis-driven studies, these resources examine the skeletal phenotype of 'unbiased' collections of mutant mouse lines, at least in terms of their relationship with established skeletal pathways. These allow the unbiased discovery of genes that have the potential to affect the skeleton and can be grouped in two classes: broad, multi-tissue phenotyping databases, such as the Mouse Genome Database (MGD, [48], http://www.informatics.jax.org) and International Mouse Phenotyping Consortium (IMPC, [49], https://www.mousephenotype.org); as well as specialised, skeleton-specific phenotyping pipelines such as the bonebase project ([50], http://bonebase.org) and the Origins of Bone and Cartilage Disease collaboration (OBCD, [51], http://www.boneandcartilage.com).

The MGD and IMPC databases contain the largest collections of genotype-phenotype data for the skeleton. MGD is a core component of the Mouse Genome Informatics (MGI) consortium which integrates a diverse range of mouse data, from gene expression to mouse-strain catalogues [48]. MGD collates, organizes phenotypic data from mouse models with spontaneous, mutagensised, and genetically engineered alleles [48]. These mice models are produced in 'stand-alone'

experiments and in systematic knockout pipelines. While it is important to note that MGD do not annotate phenotypes exclusively to genes or alleles as such, rather genotypes as the combination of alleles and mouse strain background, this resource contains phenotyping data for over 10,000 genes. These encompass both protein-coding sequences and non-coding RNAs, and identify > 1500 established mouse models of human diseases (Supplementary file 1). Aiding the extrapolation of mouse findings to humans, phenotypic associations are annotated with mammalian phenotype (MP) ontology descriptions [52]. The MP ontology provides a standardised vocabulary for the annotation of phenotypes between mammalian species. In total, 1569 genes in the MGD database (excluding conditional alleles) are annotated with one or more MP terms related to the skeleton.

The IMPC database is an extensive data resource that collates mouse phenotype data from single gene knockout animals engineered and bred at dedicated breeding and phenotyping centres from around the world [49]. These centres perform a standard phenotypic assessment of genetically engineered mouse lines as embryos, in life and terminally at 16 weeks of age. In contrast to the MGD database, the knockout mouse lines in the IMPC are produced on a standardised C57Bl6 background using standardised genetic engineering technologies. Skeletal phenotyping is based on morphological data collected by whole body dual-energy X-ray absorptiometry (DXA), with associated X-ray images also made available through the web portal (https://www.mousephenotype.org). To date, 5570 genes have been phenotyped, either by homozygous knockout, or by hetrozygous/hemizygous knockdown, in the case of embryonic lethality in the homozygote. Consistent with the frequency of skeletal phenotypes in the MGD database, ~15% of mouse lines tested in the IMPC have a phenotype association to abnormal skeleton morphology (830 of 5318 of all genes screened), defined as “any structural anomaly of the bony framework of the body in vertebrates”. It is important to note that the data from IMPC contributes to the MGD database (although periodic importing means the two resources are at times out of sync). Nevertheless, the concordance between these resources indicates a significant fraction of the total genes in the genome have the capacity to influence skeletal function and phenotype.

In addition to these broad phenotyping databases, the bonebase and OBCD projects perform detailed skeletal phenotyping of mice models following primary screening through the IMPC pipeline. The bonebase performs high-resolution morphological phenotyping by micro computed-tomography (μCT) to identify those mouse models with abnormal cortical or cancellous bone structure [50]. These knockout lines are then selected for histological analysis of bone activity, with fluorochrome labelling and histochemical staining to identify and quantify osteoblast and osteoclast surfaces, respectively. Phenotypic data for 220 mouse lines have been reported for both male and female mice, revealing a number of lines with dimorphic differences in bone volume between the sexes. This data also shows increased sensitivity to the detection of abnormal bone morphology than the DXA based primary screening pipelines, underscoring the added value of specialist skeletal screening of IMPC lines.

The OBCD skeletal phenotyping pipeline screens not only morphological phenotype but also the mechanical properties of bone [44,51]. This involves the digital x-ray micro radiography to estimate bone length and mineral content, μCT to examine bone shape and micro architecture, as well as biomechanical testing by three-point bend testing of the femur and compression testing of the vertebrae. In a pilot study of 100 knockout lines, 9 genes were identified that affected not only bone mass but also functional differences in bone strength and flexibility [51]. Among those were genes that have now been associated with human disease, or shown to be involved in bone cell function and skeletal homeostasis [53–55]. These lines demonstrate the capacity of unbiased screening approaches to identify genes relevant to skeletal homeostasis and health. To date, phenotyping data has been generated for 733 knockout lines (of a projected total of 1750) through the OBCD pipeline, making it a considerable resource to identify genes that influence skeletal morphology and function.

The genetic engineering and experimentation afforded by mice models can assist not only in the validation of suspected causal mutations, but also, through these resources, help inform causal variant prioritisation. There are, however, some important considerations associated with the interpretation of these data. Just because a whole gene knockout produces a skeletal phenotype, does not mean a specific mutation will also have a skeletal effect [46]. The effect of a specific variant is dependent on the importance of the mutated sequence to the assembly and function of the molecule it encodes. Moreover, there are instances where variants in locations within the same gene have distinct skeletal effects [56]. Gene trap constructs used in high throughput screens are designed to disable gene function by the conditional excision of critical exons [57]. Thus, the gene ‘knockout’ phenotype may not be reflective of variants with more subtle effects on gene transcription, translation or protein function. Similarly, the mouse phenotype can be strongly influenced by strain background, and thus an absence of phenotype in one strain is not conclusive evidence that a gene does not influence the skeleton [26]. Nevertheless, these resources provide *in vivo* evidence that a gene has the capacity to affect skeletal phenotype, potentially important insight in the search for causal mutations in human datasets.

4. Identifying genes that are important to the skeleton

Another layer of information which can help refine a list of gene mutations toward plausible causal candidates is the ability to associate a gene with biological process and pathways that have established physiological functions. While manual curation of gene lists in literature databases may be possible when variant lists are short, such an approach to identify genes with known skeletal function becomes impractical when faced with large numbers of genes. Alternatively, a number of resources have been developed which collate, curate and organize gene-function associations previously identified in the literature, grouping them under terms related to biological processes.

Perhaps the best known of these resources is the Gene Ontology (GO) project which annotates genes to over 40,000 biological categories based on evidence presented in over 140,000 peer-reviewed articles ([58,59], http://www.geneontology.org). The GO project gathers data from a range of *in vitro* and *in vivo* systems, with insight derived from model organisms such as *Mus musculus* used to infer orthologous gene-function relationships in other species, including humans. This resource contains 116 GO terms directly related to the skeleton, associating more than 660 genes with skeletal biological processes and cell types (~2.7% of all genes annotated within the GO database, Supplementary file 1). Among these, 11 terms are directly associated with the differentiation and function of osteoblasts and osteoclasts, respectively, and 17 terms linked to chondrocytes. Interestingly, no terms are directly associated with osteocyte cell biology, despite their important regulatory role within the [60]. This absence highlights an important knowledge gap surrounding the genes, pathways and processes important to the formation and specialised function of these critical skeletal cells.

Interestingly, there is a substantial discrepancy between the number of genes associated with skeletal functions in the GO database and those that cause significant skeletal phenotypes in identified in either the MGD databases (Fig. 2, Supplementary file 1). 379 genes associated with a significant skeletal phenotype in the MGD are annotated with a GO term directly related to skeletal functions. This indicates that more than half of the 663 genes annotated with skeletal biological processes in the GO database are supported with *in vivo* evidence of skeletal involvement. However, conversely, this represents less than a quarter of genes (1569) associated with a skeletal phenotype. This indicates that skeletal function of many genes with important regulatory roles in the skeleton are yet to be discovered.

Alongside the GO project, there are a number of other resources

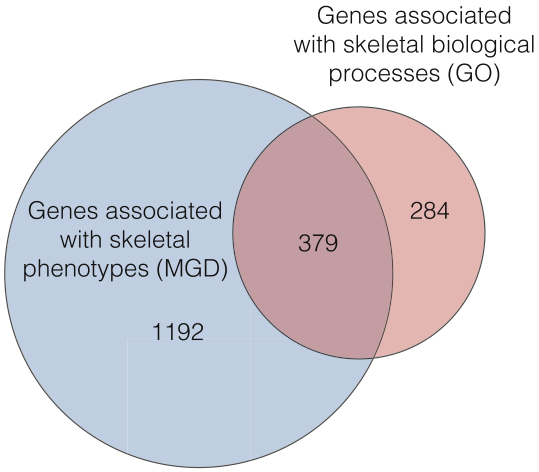

Fig. 2. Genes that are associated with skeletal phenotypes and annotated with skeletal functions. The skeletal function is not well defined for many genes that are associated with abnormal skeletal phenotypes in genetically engineered mouse models.

which can help structure our understanding of gene function. The KEGG (Kyoto Encyclopedia of Genes and Genomes, [61,62], https://www.genome.jp/kegg) and Reactome ([63], https://reactome.org) projects organize genes into functional molecular networks and biochemical pathways for a range of species, along with tools for their analysis and visualization. Using any of these resources comes with the important consideration that the constant advance of scientific knowledge means that none can be exhaustive in their gene-function annotations. Moreover, as each project has its own criteria for the evaluation of evidence required to annotate genes with molecular processes (which is catalogued for every gene annotation). As such, the researcher may want to make their own assessment of the strength of evidence supporting individual gene-function associations. Even so, these curated, literature-based gene-to-function annotations are a useful starting point to identify genes associated with skeletally significant biological processes and pathways.

Gene expression within disease-affected tissues and cell types can also be used to assess the likelihood of whether gene variants are disease-causing. In a range of organs, disease-trait heritability is seen to be enriched in regions surrounding genes with specific expression in a tissue related to the disease aetiology [64]. Many genes known to cause severe skeletal dysplasia are highly expressed in skeletal tissues and somewhat restricted to skeletal cell types [65]. Expression-based prioritisation strategies are reliant on the availability of high-quality transcriptome data from disease tissues and disease relevant cell types [64,66]. Online repositories of transcriptome data such as the Gene Expression Omnibus (GEO, [67], https://www.ncbi.nlm.nih.gov/geo) and ArrayExpress ([68], https://www.ebi.ac.uk/arrayexpress) databases contain a modest number of skeletally related, publicly available datasets (Table 1). However, the vast majority of these skeletal datasets are limited in their utility as the experimental methodologies by which they were generated are poorly described and many are under powered. As a result, the availability of quality transcriptome data from skeletal tissue and cells is limited.

Unfortunately, the skeleton is poorly represented in major initiatives to map the mammalian transcriptome, such as the GTEX, ENCODE and FANTOM projects ([39,69,70]. As a consequence, the nature of gene expression within the skeleton and how this compares to other tissues is not well defined. The GeneAtlas dataset, interrogatable through the BioGPS webportal ([71,72], http://biogps.org), contains perhaps the most comprehensive dataset of gene expression in skeletal tissues. This resource summarises transcriptome data from *in vitro* differentiated osteoblasts, osteoclasts and whole bone tissue, along with over 60 other tissue and cell types. One potential limitation associated with this dataset is that it was generated by microarray, limiting expression estimates to only those included in the assay. Microarray has in many ways been superseded by RNA sequencing (RNA-seq) technologies in the last decade [73]. In addition to gene expression quantification, which is measured by both technologies, RNA-seq returns transcript sequence information enabling the discovery on new genes and transcripts by bioinformatic transcriptome assembly [74]. Only a limited number of RNA-seq studies performed on primary skeletal tissue have been reported in the literature, and such approaches to gene discovery have been applied sparingly to the skeleton. Thus, some of the genes most restricted in expression to the skeleton may yet to be discovered.

To understand the nature of gene expression in the skeleton, examination of the transcriptome of key skeletal cell types and biological contexts is required. An important consideration when interpreting transcriptome data is that gene expression is tissue-dependent and can be strongly influenced by external factors. This includes biological contexts such as age, sex and disease state, but can also include technical factors such as sample handling and processing time. For example, Ayturk and colleagues showed that the transcriptome of osteocytes was overtly impacted by sample collection methodologies [75]. While collagenase digestion and *ex vivo* incubation are commonly used to improve sample purity [76,77], Ayturk et al. showed that such methodologies significantly alter gene expression in the remaining cells [75]. In contrast, samples isolated by fluid flushing and centrifugation retained non-skeletal cells, leading to variability in gene expression estimates. To account for this lack of purity, Ayturk et al. utilized an *in silico* gene filtering strategy to identify and remove genes whose expression was likely to be strongly influenced by contaminating cell types [75]. The optimisation of techniques in murine studies make it possible to generate transcriptome data from tissues that are hard to obtain from humans, and make it feasible to control for technical factors that influence gene expression measurements. Future studies must examine gene expression in critical skeletal cell types, with a focus on understanding not only which genes are expressed, but the specificity of gene expression relative to other tissue types, growth stages and bone types. Such datasets would provide context as to where and when genes are expressed in the skeleton, aiding in the identification of human mutations with the potential to directly affect the skeleton, as well as putative therapeutic targets.

Table 1
Publicly available transcriptome datasets from skeletal cell types.

Species	Database[a]	Chondrocyte		Osteoblast		Osteoclast		Osteocyte	
		Array	Seq	Array	Seq	Array	Seq	Array	Seq
Homo sapiens	AE	34	2	76	8	12	0	3	1
	GEO	18	8	38	8	10	3	2	1
Mus musculus	AE	30	5	66	4	22	1	6	4
	GEO	25	17	47	10	11	3	9	1

[a] Datasets may be listed in both databases. AE = ArrayExpress, GEO = Gene Expression Omnibus. Array = gene microarray, Seq = RNA sequencing.

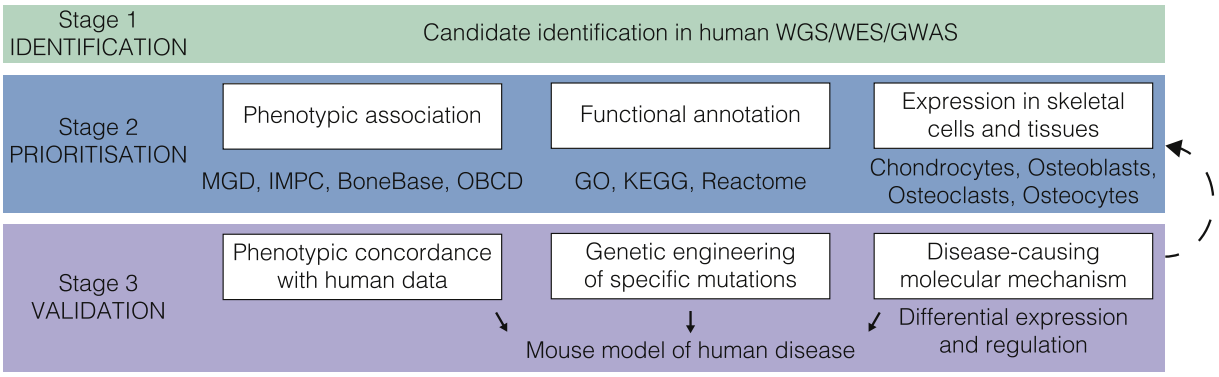

Fig. 3. Hypothetical approach to using mouse genetics to identify disease causing mutations and variants. Stage 1 - Identify candidate variants and mutations co-occurring with disease in human patients and populations. Stage 2 - Use mouse data resources to prioritise genes for further investigation based on pre-existing gene-phenotype relationships of candidate genes, their biological function, as well as their expression and regulation in skeletal tissues and cell types. Stage 3 - Generate genetically engineered mouse models of prioritised mutations to examine their specific phenotypic effects and concordance with the human disease. Leverage access to affected tissues afforded by mouse models to establish molecular mechanisms of disease. This knowledge can potentially inform disease predisposition, management and understanding of skeletal biology.

5. Prioritisation of plausible candidates - from gene lists to disease models

Above we have identified and discussed a number of resources using mouse genetics to associate genes with skeletal functions and phenotypes. Here we present a hypothetical framework outlining how these data may help inform the prioritisation of plausible causal candidates in human disease for further research (Fig. 3).

5.1. Stage 1 – Identification

The first step in the identification of causal variants and mutations requires filtering to those predicted to have a deleterious effect on gene function or regulation. Heuristic measures of importance such as mutation frequency in human populations and evolutionary conservation of base sequences may also be informative to help refine gene lists [78,79]. Additionally, patterns of inheritance and genome data from closely-related, unaffected family members can help exclude non-causal variation [80]. Databases of genes and variants with established disease relationships may be useful to screen for known causal mutations [81]. In the GWAS context, lists may constitute genes linked to loci significantly associated with the heritable variation of skeletal traits such as BMD.

5.2. Stage 2 – Prioritisation

Having excluded known causal genetic factors and filtered variants to those most likely to impact gene expression or function, it is at this stage mouse genomic data can help prioritise genes, providing additional evidence for an effect on the skeleton. In this context, data from broad phenotyping screens of gene knockouts, such as MGD and IMPC, can help identify genes with gross effects on skeletal morphology [48,49]. Further, data generated in the Bonebase or OBCD pipelines may help identify additional phenotypes not flagged in the primary screens, such as microstructural changes or changes in bone strength [50,51]. As mice from both these specialised pipelines are first processed through the primary screen, it may also possible to identify mouse models with pleiotropic effects concordant with clinical phenotype, further aiding causal gene identification.

Next, genes annotated related to skeletal biological processes or biochemical pathways can be identified using the GO, KEGG and Reactome databases [59,61,63]. It may be important to consider the strength of evidence for individual annotations of genes identified when establishing the likelihood of skeletal effect. Additionally, high-quality transcriptome datasets can be used to determine the nature of affected gene expression in skeletal tissue and cell types. Where available, data examining expression in conditions as closely matched to the patient should be used, considering factors such as age, sex and affected bone types. Such samples may be difficult to obtain from human and thus analogous data from mice may be a valuable alternative. Coupled with information of the clinical phenotype, gene expression patterns reflecting skeletal effects may inform decisions as to causal gene likelihood.

While none of these data can establish gene-disease causality, combining these layers of evidence of skeletal effect may help rationalise the selection of targets for further investigation.

5.3. Stage 3 – Validation

Once a short list of plausible causal mutations has been established, experimental evidence of their specific skeletal effect and mechanism is required to establish a disease-causing relationship. In terms of time and resources, this is a significant step, and functional genomics represents a considerable challenge in maximising our knowledge of the genetic basis for health and disease. In this context, genetically engineered mouse models simulating the specific mutation identified in the human case/cohort can provide *in vivo* evidence of disease-causing effects [13,14]. Coupled with systems biological techniques, such models can drive hypotheses into molecular mechanisms and pathways important to skeletal health. These data can feedback as evidence of skeletal association for the identification disease causing variants in skeletal pathways. Moreover, the concordance of mouse phenotype with the human clinical case can establish new disease models to help inform clinical management and therapeutic development.

6. Conclusions

In summary, while the strength of human genomic data to identify genes with potential relevance to human skeletal disease is undeniable, the task of discerning causal mutations from natural variation is far from straight forward. Mouse genetics can provide insight into gene function and the phenotypic effect of mutations, important to evaluate the likelihood that a given gene-variant causes disease and to strategize further research. Here we identify and evaluate a number of resources which use mouse genetics to help connect genes with skeletal function and phenotype. We recognize a relative scarcity of data related to gene expression in skeletal tissues and cells, not only limiting insight into the genes that are directly relevant to skeletal health, but also impeding the proper evaluation of the relevance of murine models to the study the human skeleton. Addressing these knowledge gaps will strengthen our

understanding of genes that are important to skeletal pathophysiology. As a lens to examine and tool to test the effects of mutations identified in human genetic data, mouse genomics can aid understanding of human skeletal disease.

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Conflict of interest statement

Each author certifies that he has no conflicts of interest in connection with the submitted article.

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