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Sclerostin: how human mutations have helped reveal a new target for the treatment of osteoporosis

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In the 1990s there was a tremendous mood of optimism among pharmaceutical scientists that identification of disease-associated variations in the human genome would result in a surge of new drug targets (the 'gene-to-drug' mantra). To date the expected deluge of new drugs has not arrived. However, a small number of drugs arising directly from the study of rare human disorders showing Mendelian inheritance are now entering late stage clinical trials. Here we describe the advantages of this approach and discuss the background and early clinical trial findings with antibodies directed at a target identified in this way.

Introduction

Studies comparing disease concordance in mono- and dizygotic twins have shown that both genetics and environmental factors are important in the development of many common disease states [1]. The inheritance pattern of many of these disorders is complex and genome wide association studies (GWAS) have helped to identify a large number of gene variants that are linked with one or more human diseases [2]. However, many of the gene variants identified by GWAS only marginally increase the risk of an individual developing the disease. This information is useful to drug developers in illuminating the wide variety of pathways that can contribute to a disease phenotype but for many common conditions it would take tens if not hundreds of such weakly disease-associated gene variants to account for all of the 'genetic risk' of developing the disorder [2]. For pharmaceutical scientists these weak links between a gene variant and a disease do little to provide the level of target validation needed to support a drug discovery project.

In contrast to the weak disease associations uncovered by GWAS several rare inherited diseases show clear Mendelian (monogenic) patterns of inheritance where the presence of a mutant gene (or two mutant alleles of a gene in the case of recessive inheritance) results in the development of the disease phenotype. In such cases there is a clear link between the mutant gene and the phenotype. For highly penetrant monogenic diseases it seems probable that the phenotype resulting from a gene-inactivating mutation will provide a preview of the effects of an inhibitory drug directed at the same gene product in the general population (excluding developmental effects). Similarly, the phenotype of a gain-of-function mutation should be mimicked by an activating drug (although these are less common). A large number of human disorders showing Mendelian inheritance have been identified and the phenotypes and mutant genes are catalogued in databases such as OMIM (Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/omim — Box 1). Many of these rare conditions are severe and/or disabling and some may be candidates for gene replacement therapy. While the majority of such conditions do not provide obviously useful information about new therapeutic targets, in a small number of disorders the rare genetic defect disrupts a previously unknown pathway, thus revealing a new target for therapeutic intervention in a more common disorder. When considering these rare disorders it is important to remember that a detrimental phenotype from one disorder can be therapeutically helpful if replicated in a different disease state. For example, patients with Glanzmann thrombasthenia have bleeding diathesis due a defect in a gene that encodes a platelet integrin [3]. The integrin in question (gpIIbbeta3) has been targeted with drugs

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BOX 1

Useful database links

OMIM (Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), URL: http://omim.org/) is a database of human genes and genetic phenotypes that was initiated in the 1960s by Dr V McKusick. It currently lists more than 3600 Mendelian phenotypes for which the causative mutation is known and over 1700 Mendelian phenotypes for which cause is unknown. ClinVar (http://preview.ncbi.nlm.nih.gov/clinvar/) was recently established by the NIH and NCBI and is an integrated resource that brings together information from several existing databases on human genomic variation and phenotypes.

The LOVD (Leiden Open (source) Variation Database) provides information on known DNA sequence variations. Information on variants of the *SOST* gene can be found at: http://chromium.liacs.nl/LOVD2/home.php?select_db=SOST.

ClinicalTrials.gov is a worldwide registry of clinical studies (http://clinicaltrials.gov/ct2/search). Details of clinical trials using AMG785 (Romosozumab) can be found at: http://clinicaltrials.gov/ct2/results?term=AMG785.

such as tirofiban to act as anticoagulants in acute coronary syndromes [4]. The logic of using information obtained from the study of human monogenic conditions to identify useful new drug targets is compelling, particularly because the approach does not involve extrapolating phenotypes across species. However, identifying inherited diseases which provide useful information for new drug targets requires both insight and imagination. Here we describe how the study of a rare monogenic high bone mass disorder called sclerosteosis has provided a new insight into the regulation of bone formation.

The sclerosteosis phenotype

Sclerosteosis (OMIM 269500) is a rare condition characterised by high bone mass that is inherited in an autosomal recessive manner [5]. Persons with sclerosteosis are found predominantly in the Afrikaner community of South Africa, although a few cases have been reported elsewhere in the world [5]. At first sight sclerosteosis appears an unpromising disorder to provide information on a new drug target. Manifestations of the condition include large stature, mandibular overgrowth and distortion of the facial features. Syndactyly, usually of the 2nd and 3rd fingers is a syndromic component. Progressive bone overgrowth in the skull leads to constriction of the foramina of the 7th and 8th cranial nerves resulting in facial palsy and deafness. Thickening and widening of the calvarium cause potentially lethal elevation of intracranial pressure which develops in early adulthood [6]. From a drug discovery perspective a more interesting feature of the phenotype is revealed by radiographs which show that affected persons have abnormally large and dense bones [7]. It has become clear that aberrant regulation of bone formation in these individuals underlies all the characteristic features of the condition except for syndactyly. The syndactyly present at birth is a consequence of defective embryogenesis; the other complications all develop after birth as a result of excessive bone accumulation.

Several human high bone mass conditions have been recorded (e.g. pycnodysostosis, and the autosomal dominant and recessive forms of osteopetrosis); while all of these conditions are associated with excessive bone they are also associated with an increased risk of bone fracture [8], suggesting that the underlying mutations have compromised bone quality. One of the interesting features of sclerosteosis is that it is not associated with an increased risk of fracture. In fact, there is anecdotal evidence that individuals with sclerosteosis are resistant to fracture; Hamersma *et al.* [6] note in their review of the condition that there has never been a recorded traumatic fracture in an affected individual. Early biomarker and histomorphometric analysis of samples from a small number of individuals affected with sclerosteosis by Stein and colleagues [9] revealed an interesting insight into the nature of the mutation. They noted that affected individuals showed signs of excessive activity from bone forming osteoblasts with near normal (or slightly reduced) activity from bone resorbing osteoclasts [9]. Hamersma et al. [6] analysed their findings in a cohort of 63 persons with sclerosteosis studied in South Africa over a 38-year period. They stated that there was no obvious clinical or radiological evidence that the condition affected either the central or peripheral cardiovascular system or promoted endocrine disturbance. Similarly, they reported that the disorder was not associated with ectopic bone formation or degenerative osteoarthropathy [6]. These observations provided some reassurance that the defective gene did not have extensive pleiotropic effects.

The genetics of sclerosteosis was further defined through careful assessment of bone mineral density (BMD) in affected individuals and gene carriers [10]. As expected, the affected individuals had markedly increased BMD at all skeletal sites. Interestingly, BMD in heterozygous carriers was above the mean value of healthy age-matched individuals at all skeletal sites; this increased BMD apparently has no pathological consequences but does suggest that the skeletal system is exquisitely sensitive to the absolute amounts of the gene product that is affected in this disorder.

The sclerosteosis phenotype, together with biochemical and histomorphometric study results described above, suggested that the genetic mutation up-regulates osteoblast activity to increase normal bone formation without affecting other physiological systems. Although this conclusion was based on extrapolations from a limited amount of information, it offered the possibility that the gene which is defective in patients with sclerosteosis encodes a novel regulator of bone formation with very specific activity that was amenable to pharmaceutical manipulation, that is, a potential new drug target for treating low bone mass disorders such as osteoporosis Fig. 1.

Osteoporosis

Most people reach peak bone mass in their mid-20s and then undergo an age-related and generally undetected decline in bone quantity and quality which often leads to bone fragility and osteoporosis [11]. The effects on the lumbar spine of osteoporotic bone-loss are shown in Fig. 2. Bone fractures in the elderly are associated with high morbidity and mortality [12] as well as placing a high financial burden on health care systems. It was estimated that the cost to the US economy from osteoporosis and fractures was $22 billion in 2008 [13]. Several drugs (e.g. bisphosphonates, SERMs, RANKL inhibitors) were developed to combat bone loss but these therapies are less effective at restoring bone mass that has already been lost [14]. This has stimulated

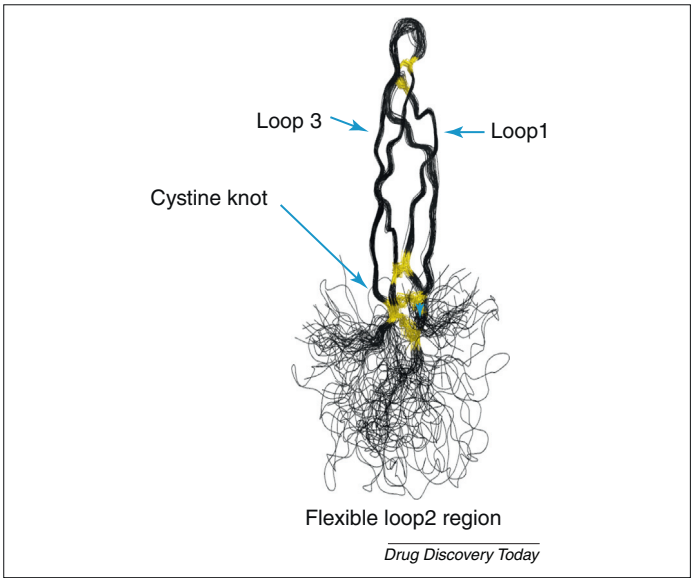

FIGURE 1

Solution structure of the core region of sclerostin shows a best-fit superposition of the protein backbone for a family of converged structures (determined by NMR) of the central region of sclerostin (the unstructured N- and C-terminal arms have been omitted). Three loops arise from the central cystine knot, two of the loops (loops 1 and 3) are rigid (made up of twisted anti-parallel beta strands joined by a disulphide bond at their tips) while the remaining loop (loop 2) is flexible [33] and is involved in binding to LRP5/6 [36]. Figure by kind permission of Dr Vaclav Ververka, Institute of Organic Chemistry and Biochemistry, Prague Czech Republic.

considerable interest in searching for new therapies that effectively promote bone formation.

Molecular basis of sclerosteosis

The largest number of individuals affected by sclerosteosis reside within the Afrikaner population in South Africa. Because of the relative ethnic isolation of the affected families, as well as the extreme rarity of the disorder throughout the rest of the world, it was presumed that a single recessive founder mutation was responsible for all Afrikaner cases. In addition, Beighton [5] had hypothesized a direct link between sclerosteosis in the Afrikaners and van Buchem disease in a small Dutch ethnic isolate. Van Buchem disease (OMIM 293100), like sclerosteosis, is a sclerosing bone dysplasia with many of the same features as sclerosteosis; it is, however, generally less severe and syndactyly is never observed. Because of the shared Dutch ancestry and very similar phenotypes, Beighton predicted that a single genetic lesion would be responsible for the disorders in the two different populations; subtle phenotypic differences would then need to be explained by epigenetic factors. The hypothesis was strengthened by reports that sclerosteosis and van Buchem disease localized to overlapping regions of chromosome 17 [15,16]. Brunkow *et al.* [17] used homozygosity mapping and positional cloning to determine the molecular basis of sclerosteosis in the Afrikaner population — a previously unknown gene carrying a chain terminating mutation in its presumptive signal sequence. The same candidate gene, named *SOST*, was identified in a small number of other consanguineous families and unrelated individuals affected with sclerosteosis [17,18]. Several mutations in the SOST gene were subsequently identified (see Box 1). Surprisingly, no mutations

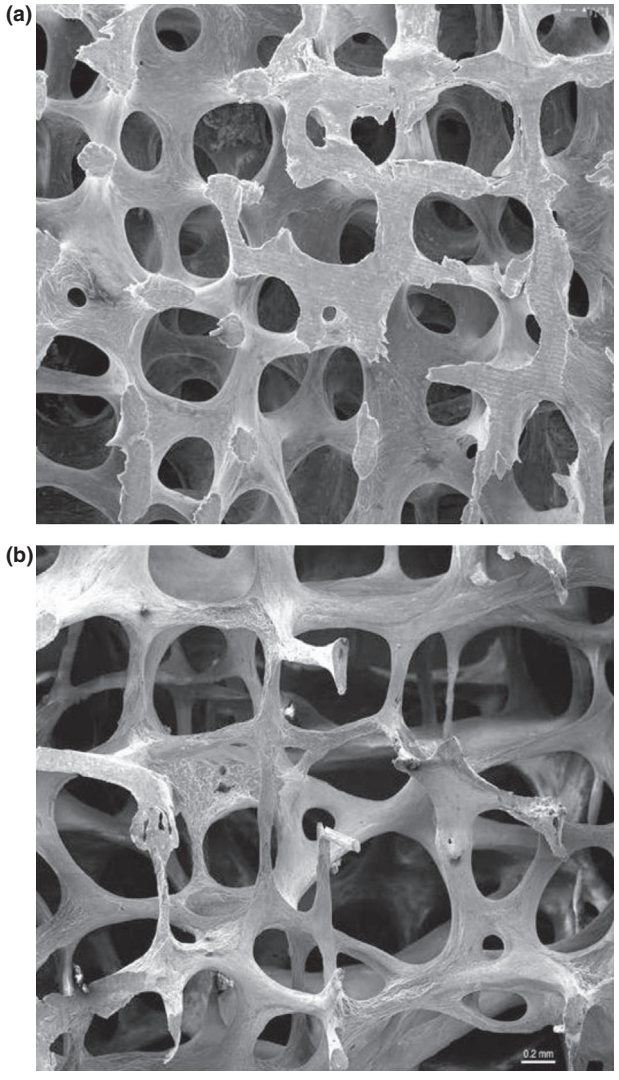

FIGURE 2

Vertebrae from individuals with and without osteoporosis Sections through the 3rd lumbar vertebrae from an individual with (a) normal and (b) osteoporotic bone. The osteoporotic bone shows signs of bone thinning and pits caused by osteoclastic bone resorption are visible. Picture by kind permission of Prof Tim Arnett, University College, London.

in *SOST* were found in families with van Buchem disease, thus disproving the hypothesis of a common Dutch founder mutation.

The role of serendipity in the identification of sclerostin

It is interesting to reflect on how luck can play a part in target discovery. Starting with Afrikaner families affected with sclerosteosis, Brunkow *et al.* [17] were able to identify the loss-of-function mutation in the *SOST* gene, which encodes a small glycoprotein called sclerostin [17,18]. In this case the link between the phenotype and the mutant gene was clear.

After the causative mutation in sclerosteosis had been identified, two groups [19,20] identified the causative genetic lesion in van Buchem disease. The lesion turned out to be a 52 kb DNA deletion located 35 kb downstream of the gene encoding sclerostin. Without prior knowledge of the mutation in sclerosteosis the

link between the deletion and the gene encoding sclerostin would not have been obvious. In other words, if the initial mapping and sequencing had been performed in the population affected with van Buchem disease rather than in the sclerosteosis cohort, the discovery of sclerostin as a new drug target may have been delayed for several years. Subsequently, it has been shown that the DNA deletion in van Buchem disease removes an enhancer region which is necessary for post-embryonic sclerostin expression [21]. The enhancer is not required for correct expression of sclerostin in the embryo, which explains the lack of syndactyly in individuals with van Buchem disease. A recent report indicates that low levels of circulating sclerostin can be detected in almost all adults affected with van Buchem disease [22], indicating that the deletion in these patients is a hypomorphic mutation, which may explain why their phenotype is less severe than those affected with sclerosteosis (where sclerostin is completely absent).

Human genetics and sclerostin's mode of action

Bone is a dynamic tissue with continuous cycles of bone resorption (mediated by osteoclasts) and bone formation (mediated by osteoblasts). Regulation of resorption and formation allows bone to adapt to changes in loading and to repair stress-induced cracks [23]. Bone formation and resorption are regulated by cells called osteocytes living within a network of fluid-filled canals in the bone [24]. Sclerostin is an osteocyte-derived protein that signals to osteoblasts to reduce bone formation [25]. Thus the overproduction of bone seen in sclerosteosis is the result of the loss, through genetic mutation, of this important braking system. Based on an analysis of its amino acid sequence, sclerostin was initially recognised as a secreted cystine knot protein most closely related to the dan/cerberus family of proteins [17] which regulate bone morphogenic proteins (BMPs). Consistent with this, sclerostin was shown to bind to BMPs and inhibit their signalling [26,27]. Very soon after the reports identifying mutant sclerostin as the cause of sclerosteosis two groups reported that point mutations in a Wnt co-receptor called LRP5 led to a human high bone mass phenotype which was similar to the sclerosteosis phenotype [28,29]. At the time, a direct link between these two genetically distinct conditions was not obvious. In the meantime, further investigation into the function of sclerostin suggested that it did not behave in the same way as classical BMP antagonists such as noggin, but rather may actually antagonise a BMP-inducible factor [30]. Li *et al.* [31] subsequently showed that sclerostin inhibited Wnt signalling by binding to LRP5/6 and that LRP5 containing the high bone mass mutation bound less well to sclerostin than wild type LRP5. As evidence mounted that sclerostin was a Wnt antagonist it was even suggested that sclerostin did not directly inhibit BMP activity [32]. This dispute appears to have been settled in 2010 when Krause et al. [33] confirmed that sclerostin could both inhibit Wnt signalling and bind intracellularly to BMPs to prevent secretion of active BMP protein.

The structure of sclerostin determined by NMR has been reported by two groups [34,35]. Sclerostin has unstructured N- and C-terminal arms and a central cystine knot motif Fig. 1. The way in which sclerostin interacts with BMPs is currently unknown but the interaction of sclerostin with LRP5/6 has been shown to involve part of its flexible loop2 region [36,37]. Mutations in LRP4 (another member of the LRP family of receptors) have also been found to be associated with a high bone mass phenotype in humans [38], which again resembles sclerosteosis; and it has been shown that sclerostin also interacts with this receptor. As with LRP5, the high bone mass mutations in LRP4 interfere with its interaction with sclerostin [38,39], thus LRP4 appears to potentiate the ability of sclerostin to inhibit Wnt signalling.

Sclerostin activity *in vivo*

Mesenchymal stem cells (MSC) are the precursors of bone regulatory osteocytes. Detailed studies have shown that in the pathway of MSC differentiation to osteocytes the late osteoblast cells are the most sensitive to sclerostin activity [40]. Sclerostin inhibits the terminal differentiation of late osteoblasts into osteocytes and the accompanying mineral deposition. It has been suggested that one function of sclerostin may be to upregulate the formation of proteins in the Small Integrin-Binding Ligand, N-linked Glycoprotein (SIBLING) family (e.g. Matrix Extracellular Phosphoglycoprotein, or MEPE). These proteins contain acidic serine-aspartate-rich motifs (i.e. ASARM peptides) that bind to freshly mineralised surfaces and inhibit further mineralisation. Sclerostin also seems to inhibit the formation of PHEX, a metalloprotease that inactivates ASARM peptides [40] and prevents them from disrupting mineralisation. Mice deficient in sclerostin largely recapitulate the high bone mass phenotype seen in humans with sclerosteosis [41]. In several preclinical models (including rodents and non-human primates) antibodies to sclerostin have been shown to increase bone formation, bone mass and bone strength. Much of this work has been previously reviewed by Paszty *et al.* [42] and Ke *et al.* [43].

Human studies with antibodies to sclerostin

In 2010 Padhi *et al.* [44] reported a first in human study with a humanised monoclonal antibody targeted at sclerostin (AMG785/CDP785 — recently assigned the name romosozumab). The single dose of antibody administered in this study mediated a pronounced increase in markers of bone formation. P1NP (a circulating marker of bone formation generated by the processing of type 1 collagen) showed a dose-dependent increase with peak levels of the biomarker being attained 2–3 weeks after dosing (with more than a 120% increase of P1NP levels above baseline when dosed at 5 mg/kg subcutaneously). Similar profiles were seen with other markers of bone formation. Subjects dosed with romosozumab also showed an approximately dose-dependent decrease in a circulating marker of bone resorption. This was of significance because it showed that the drug had disassociated the normally tight relationship found in adults between bone formation and bone resorption. Studies in both rodents and non-human primates [45,46] have also suggested that treatment with an antibody to sclerostin activates osteoblastic bone formation without the usual requirement for a preceding phase of osteoclastic bone resorption. A recent report by Wijenayaka *et al.* [47] reported that sclerostin caused an upregulation of RANKL (an activator of osteoclast activity) and inhibition of OPG (an inhibitor of osteoclast activity) in cultures of osteocyte-like cells. This observation provides an explanation

for the decrease in bone resorption associated with romosozumab administration.

Two other studies with antibodies to sclerostin were reported at the 2012 American Society of Bone and Mineral Research (ASBMR) meeting. The first was a multiple-dose phase 1 study with a humanised monoclonal antibody to sclerostin called blosozumab [48]. In this study the antibody was administered at a range of different doses up to 5 times. No serious safety concerns were reported and by day 85 of the study subjects receiving multiple doses of the drug showed up to a 7.7% increase in BMD at the lumbar spine. The second report at the 2012 ASBMR meeting described results from a phase 2 study with romosozumab [49]; again the drug was well tolerated with no new or serious safety concerns. In this study the highest monthly dose (210 mg) provided an increase in BMD, as measured by DXA, of 11.3% at the lumbar spine and 4.1% at the total hip after one year.

Did the sclerosteosis phenotype accurately predict the outcome of clinical studies with antibodies to sclerostin?

The published clinical studies with sclerostin antibodies have all shown that they induce an increase in bone mass and bone formation, consistent with the phenotype seen in individuals that are congenitally deficient in sclerostin (i.e. individuals with sclerosteosis). The increased bone formation (as measured by increased P1NP) was also predicted from biomarker studies done by Stein and colleagues in 1983 [9] on samples from sclerosteosis patients. They reported 'the intermittent and persistent elevation' of the bone formation biomarker alkaline phosphatase in some of their sclerosteosis patients. Furthermore they suggested that because osteoclast activity was not elevated in these patients there may be some alteration in the coupling of bone formation and bone resorption. However, studies on individuals with sclerosteosis did not detect the mild decrease in bone resorption seen after dosing with sclerostin antibody; this reduction may be masked by the large increase in bone formation associated with sclerosteosis. It is worth noting that all of the clinical studies published to date report that the anti-sclerostin drugs are well tolerated, consistent with the apparent bone-specific nature of the sclerosteosis phenotype. Although the current clinical studies have been too short to assess any long-term risks associated with exposure to antibodies directed at sclerostin, no issues, apart from those associated with bone overgrowth, have been reported in sclerosteosis patients.

Current status of antibodies to sclerostin

The genetic validation of sclerostin's role as a key regulator of human bone formation has made this molecule of considerable interest to the pharmaceutical industry and to physicians with an interest in the osteoporoses. This interest has been heightened because of sclerostin's limited tissue distribution (it is expressed predominantly in bone dwelling osteocytes) and because it is a secreted protein, thus making it a suitable target for monoclonal antibodies. ClinicalTrials.gov (see Box 1) lists several antibodies targeting sclerostin that are currently being evaluated in human clinical trials; AMG785 (also known as romosozumab, Amgen/UCB), AMG167 (Amgen/UCB), BPS804 (Novartis) and LY2541546 (also known as blosozumab, Eli Lilly). Of these, romosozumab is the most advanced in clinical development; it entered a phase 3 study (the FRAME study) in March 2012 with an estimated completion date in 2015. Other phase 3 studies with this antibody are also on going (see Box 1).

Identifying targets via human genetics

The sclerostin story illustrates how a rare human genetic mutation can help to identify a new drug development pathway for treating a common human disorder. As hoped, the phenotype of the sclerosteosis patients predicted important aspects of the profiles of sclerostin antibody therapeutics. While this genetic approach to identifying new drug targets is attractive, there is no guarantee that it will actually lead to a 'druggable' target. In the past (including the period during which the sclerosteosis mutation was identified) this was a major cause for concern because of the significant genetic resources, as well as high level of effort and ingenuity required to finally identify a disease-causing genetic variant. The completion of the human genome sequence, the availability of reference genomes to help recognise silent allelic variants, and the advent of next generation sequencing have combined to dramatically reduce the amount of effort required to identify a new human disease-causing mutation. Need *et al.* [50] recently reported that by only sequencing the exomes of trios (i.e. affected individual plus parents) they were able to identify credible candidate mutations in around 50% of the cases they examined. In light of these advances the main challenge today is identifying informative human phenotypes. There is little doubt that such phenotypes exist — it has been estimated that the per generation base pair mutation rate in humans is around 1×10^{-8} [51] and Frazer *et al.* [2] have noted that 'any base pair that, when altered, is compatible with life is likely to be found in at least one of the ~6.7 billion people on earth.' The pilot phase of the 1000 genomes project suggested that each human genome is heterozygous for 50–100 sequence variants that have been identified by the Human Gene Mutation database (http://www.hgmd.cf.ac.uk/ac/index.php) as potentially causing inherited disorders. This helps to explain the high frequency of recessive inherited disorders seen in areas where consanguinity is common. Rare human gene variants have already been used to identify several gene products that are currently being explored for their therapeutic potential such as the Nav1.7 sodium channel (the deficiency of which causes the complete inability to sense pain [52]), PCSK9 (the deficiency of which leads to low levels of LDL cholesterol [53]), and cholesterol ester transfer protein (the deficiency of which causes hyperalphalipoproteinemia [54]).

Conclusions

While rare human inherited diseases often represent a tragedy at a personal and family level, some may hold the key to a new generation of targets for treating common human diseases. Recent advances in DNA sequencing technology and our improved understanding of natural sequence variation in the human genome have dramatically simplified the technical difficulties involved in this genetic approach to target discovery. Until recently, drug discovery outpaced our understanding of human monogenic disease and many human mutations served only to retrospectively validate drugs in development. With our increased understanding of human genetic variation and advances in sequencing technology it seems probable that human genetics will play an ever increasing role in new target identification. The challenge for drug-hunters

today is to identify inherited phenotypes that can inform us about the next generation of drug targets.

Conflict of interest statement

MKR and JC are employed by UCB Pharma and hold stock/stock options in UCB Pharma. UCB Pharma in collaboration with Amgen Inc. is developing therapies that target sclerostin.

MEB was employed by a UCB Pharma legacy company at the time of the discovery of sclerostin and has subsequently carried out paid consultancy work for UCB Pharma.

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References

1 Bogdanos, D.P. *et al.* (2012) Twin studies in autoimmune disease: genetics, gender and environment. *J. Autoimmun.* 38, J156–J169
2 Frazer, K.A. *et al.* (2009) Human genetic variation and its contribution to complex traits. *Nat. Rev. Genet.* 10, 241–251
3 Coller, B.S. and Shattil, S.J. (2008) The GPIIb/IIIa (integrin αIIbβ3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood* 112, 3011–3025
4 Scharf, R.E. (2012) Drugs that affect platelet function. *Semin. Thromb. Hemost.* 38, 865–883
5 Beighton, P. (1988) Sclerosteosis. *J. Med. Genet.* 25, 200–203
6 Hamersma, H. *et al.* (2003) The natural history of sclerosteosis. *Clin. Genet.* 63, 192–197
7 Beighton, P. *et al.* (1976) The radiology of sclerosteosis. *Br. J. Radiol.* 49, 934–939
8 Stark, Z. and Savarirayan, R. (2009) Osteopetrosis. *Orphanet. J. Rare Dis.* 4, 5
9 Stein, S.A. *et al.* (1983) Sclerosteosis: neurogenetic and pathophysiologic analysis of an American kinship. *Neurology* 33, 267–277
10 Gardner, J.C. *et al.* (2005) Bone mineral density in sclerosteosis; affected individuals and gene carriers. *J. Clin. Endocrinol. Metab.* 90, 6392–6395
11 Clarke, B.L. and Khosla, S. (2010) Physiology of bone loss. *Radiol. Clin. North Am.* 48, 483–495
12 Johnell, O. and Kanis, J.A. (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos. Int.* 17, 1726–1733
13 Blume, S.W. and Curtis, J.R. (2011) Medical costs of osteoporosis in the elderly Medicare population. *Osteoporos. Int.* 22, 1835–1844
14 Kawai, M. *et al.* (2011) Emerging therapeutic opportunities for skeletal restoration. *Nat. Rev. Drug Discov.* 10, 141–156
15 Van Hul, W. *et al.* (1998) Van Buchem disease (hyperostosis corticalis generalisata) maps to chromosome 17q12-q21. *Am. J. Hum. Genet.* 62, 391–399
16 Balemans, W. *et al.* (1999) Localization of the gene for sclerosteosis to the van Buchem disease-gene region on chromosome 17q12-q21. *Am. J. Hum. Genet.* 64, 1661–1669
17 Brunkow, M.E. *et al.* (2001) Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. *Am. J. Hum. Genet.* 68, 577–589
18 Balemans, W. *et al.* (2001) Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum. Mol. Genet.* 10, 537–543
19 Staehling-Hampton, K. *et al.* (2002) A 52-kb deletion in the SOST-MEOX1 intergenic region on 17q12-q21 is associated with van Buchem disease in the Dutch population. *Am. J. Med. Genet.* 110, 144–152
20 Balemans, W. *et al.* (2002) Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. *J. Med. Genet.* 39, 91–97
21 Loots, G.G. *et al.* (2005) Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res.* 15, 928–935
22 Van Lierop, A.H. *et al.* (2012) Van Buchem disease: clinical, biochemical and densitometric features of patients and disease carriers. *J. Bone Miner. Res.* 1794http://dx.doi.org/10.1002/jbmr
23 Robling, A.G. *et al.* (2006) Biomechanical and molecular regulation of bone remodeling. *Annu. Rev. Biomed. Eng.* 8, 455–498
24 Thompson, W.R. (2012) Mechanical regulation of signaling pathways in bone. *Gene* 503, 179–193
25 Poole, K.E. *et al.* (2005) Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J.* 19, 1842–1844
26 Kusu, N. *et al.* (2003) Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. *J. Biol. Chem.* 278, 24113–24117
27 Winkler, D.G. *et al.* (2003) Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J.* 22, 6267–6276
28 Boyden, L.M. *et al.* (2002) High bone density due to a mutation in LDL-receptor-related protein 5. *N. Engl. J. Med.* 346, 1513–1521
29 Little, R.D. *et al.* (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am. J. Hum. Genet.* 70, 11–19
30 van Bezooijen, R.L. (2004) Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J. Exp. Med.* 199, 805–814
31 Li, X. *et al.* (2005) Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J. Biol. Chem.* 280, 19883–19887
32 van Bezooijen, R.L. *et al.* (2007) Wnt but not BMP signaling is involved in the inhibitory action of sclerostin on BMP-stimulated bone formation. *J. Bone Miner. Res.* 22, 19–28
33 Krause, C. *et al.* (2010) Distinct modes of inhibition by sclerostin on bone morphogenetic protein and Wnt signaling pathways. *J. Biol. Chem.* 285, 41614–41626
34 Veverka, V. *et al.* (2009) Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. *J. Biol. Chem.* 284, 10890–10900
35 Weidauer, S.E. *et al.* (2009) NMR structure of the Wnt modulator protein Sclerostin. *Biochem. Biophys. Res. Commun.* 380, 160–165
36 Bourhis, E. *et al.* (2011) Wnt antagonists bind through a short peptide to the first β-propeller domain of LRP5/6. *Structure* 19, 1433–1442
37 Holdsworth, G. *et al.* (2012) Characterization of the interaction of sclerostin with the low density lipoprotein receptor-related protein (LRP) family of Wnt co-receptors. *J. Biol. Chem.* 287, 26464–26477
38 Leupin, O. *et al.* (2011) Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. *J. Biol. Chem.* 286, 19489–19500
39 Choi, H.Y. *et al.* (2009) Lrp4, a novel receptor for Dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover in vivo. *PLoS ONE* 4, e7930
40 Atkins, G.J. *et al.* (2011) Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism. *J. Bone Miner. Res.* 26, 1425–1436
41 Li, X. *et al.* (2008) Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J. Bone Miner. Res.* 2008 (23), 860–869
42 Paszty, C. *et al.* (2010) Sclerostin: a gem from the genome leads to bone-building antibodies. *J. Bone Miner. Res.* 25, 1897–1904
43 Ke, H.Z. *et al.* (2012) Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. *Endocr. Rev.* 33, 747–783
44 Padhi, D. *et al.* (2011) Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J. Bone Miner. Res.* 26, 19–26
45 Ominsky, M.S. *et al.* (2010) Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J. Bone Miner. Res.* 25, 948–959
46 Li, X. *et al.* (2011) Increased bone formation and bone mass induced by sclerostin antibody is not affected by pretreatment or cotreatment with alendronate in osteopenic, ovariectomized rats. *Endocrinology* 152, 3312–3322
47 Wijenayaka, A.R. , Kogawa, M. , Lim, H.P. , Bonewald, L.F. and Findlay, D.M. *et al.* (2011) Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS ONE* 6 (10), e25900http://dx.doi.org/10.1371/journal.pone.0025900
48 McColm, J. *et al.* (2012) Blosozumab, a humanised monoclonal antibody against sclerostin, demonstrated anabolic effects on bone in postmenopausal women. *J. Bone Miner. Res.* 27 (Suppl 1), abstract 1026. Available at: http://www.asbmr.org/AnnualMeeting/2012/Abstracts.aspx

49 McClung, M.R. *et al.* (2012) Inhibition with AMG 785 in postmenopausal women with low bone density: phase 2 trial results. *J. Bone Miner. Res.* 27 (Suppl 1), abstract 1025. Available at: http://www.asbmr.org/AnnualMeeting/2012/Abstracts.aspx
50 Need, A.C. *et al.* (2012) Clinical application of exome sequencing in undiagnosed genetic conditions. *J. Med. Genet.* 49, 353–361
51 The 1000 Genomes Project Consortium, (2010) A map of human genome variation from population scale sequencing. *Nature* 467, 1061–1073
52 Cox, J.J. *et al.* (2006) An SCN9A channelopathy causes congenital inability to experience pain. *Nature* 444 (7121), 894–898
53 Cohen, J. *et al.* (2005) Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat. Genet.* 37, 161–165
54 Brown, M.L. *et al.* (1989) Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature* 342, 448–451