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Pathophysiology of Skeletal Disease

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CONTRIBUTION OF SUBMITTED PUBLICATIONS TO FIELD OF STUDY

A) Thyroid Hormones and Bone

After completing general medicine and endocrinology training in Birmingham in 1990, I moved to Harvard Medical School to undertake laboratory training in biochemistry and molecular biology studying how thyroid hormone (T3) regulates gene transcription. I returned to the University of Birmingham as a Medical Research Council (MRC) Clinician Scientist Fellow in 1993 to set up a new laboratory investigating the physiology and mechanisms of T3 action in the skeleton. It had been known for decades that untreated thyrotoxicosis causes accelerated bone loss, osteoporosis and fractures. However, little was known about the pathogenesis of bone loss in patients with thyrotoxicosis and the molecular basis of T3 action in bone had never been investigated. Thus, I had the opportunity to develop a new field of research. Initial work revealed expression and activity of T3 receptors (TRs) in skeletal cells, and I moved to a Senior Lectureship at the Royal Postgraduate Medical School (now Imperial College London) in 1995 to establish a laboratory using *in vivo* approaches to study mechanisms of T3 action in the skeleton.

1. Experiments in primary cultured cartilage-forming chondrocytes and bone-forming osteoblasts demonstrated their T3 responsiveness and led to my cloning of two new TR isoforms from osteoblastic cells. In these studies, I generated an osteoblast cDNA library and cloned two new TR variants by 5'-Rapid Amplification of cDNA Ends (5'-RACE) and inverse reverse transcription-polymerase chain reaction (inverse RT-PCR). I subsequently isolated a genomic clone and demonstrated that the two new variants, TRβ3 and TRΔβ3, arise via transcription from a novel promoter in the *Thrb* gene and alternative mRNA splicing. I next showed that both variants are also expressed widely in extra-skeletal tissues and that the relative ratio of TRβ3:TRΔβ3 expression varies between tissues and in response to changes in thyroid status. Expression and functional analysis of the TRβ3 and TRΔβ3 proteins revealed TRβ3 binds DNA and hormone and acts as a functional T3 receptor, whereas TRΔβ3 lacks DNA binding activity and acts as a dominant-negative antagonist. In addition, tissue-specific and hormone-responsive variation in levels of expression of the two isoforms regulated T3 responses in transfection studies, thus identifying a new level of control that determines thyroid status in cells expressing both TRβ3 and TRΔβ3. Overall, this work reveals new insight into the control of T3 action in specific target cells, including bone-forming osteoblasts **(Williams GR, *Mol. Cell. Biol.* 2000)**.

2. Hypothyroidism in children results in developmental abnormalities of the skeleton and growth delay, whereas thyrotoxicosis during childhood accelerates linear growth and advances bone age. Despite these well-known clinical observations, the mechanisms by which thyroid hormones regulate linear growth and skeletal development were poorly understood. Skeletal development proceeds mainly via endochondral ossification, in which mineralised bone develops on a cartilage scaffold. Linear growth occurs when growth plate chondrocytes progress through an organised programme of maturation, proliferation and differentiation that results in formation of hypertrophic chondrocytes. Cell culture studies from my own laboratory and others had shown that T3 regulates chondrocyte differentiation *in vitro*, and it was known that the skeleton in hypothyroid patients is exquisitely sensitive to thyroid hormone replacement. To determine the role of thyroid hormones in endochondral ossification *in vivo*, we studied (i) euthyroid, (ii) hypothyroid, (iii) thyrotoxic, and (iv) hypothyroid-thyroxine-treated rats. Growth plates in hypothyroid rats were grossly disorganised; proliferating chondrocytes failed to form discrete columns of cells; and the zone containing hypertrophic chondrocytes was diminished and morphologically indistinct. The growth plate was separated from underlying primary spongiosum by an abnormally mineralised layer of matrix in which invasion by new blood vessels and osteoblasts was diminished. In hypothyroid rats, alcian blue critical electrolyte staining of growth plate cartilage matrix demonstrated an abnormal preponderance of mucopolysaccharides composed mainly of chondroitin sulphate and hyaluronic acid. Islands of cartilage were also retained in primary spongiosum trabecular bone. These abnormalities were accompanied by delayed linear growth and reveal disrupted endochondral ossification with failure of hypertrophic chondrocyte differentiation and abnormal bone formation. To investigate underlying mechanisms, we performed *in situ* hybridisation analysis of the Indian hedgehog (Ihh)/parathyroid hormone-related protein (PTHrP) feedback loop, which regulates endochondral bone formation and the pace of hypertrophic chondrocyte differentiation. In hypothyroid rats with disrupted ossification,

growth plate expression of PTHrP, which acts via its receptor (PTHrP-R) to inhibit chondrocyte differentiation, was markedly increased and accompanied by undetectable expression of the hypertrophic marker, collagen X. In thyrotoxic rats, collagen X was expressed in differentiating hypertrophic chondrocytes, whereas PTHrP-R was undetectable. In addition, TRs were localised to reserve zone progenitor cells and proliferating chondrocytes in growth plates from euthyroid animals; regions in which PTHrP and PTHrP-R expression were affected by thyroid status. Overall, these studies showed that T3 regulates endochondral ossification and growth via effects on the Ihh/PTHrP feedback loop. This pathway may be a key mechanism accounting for growth retardation and delayed skeletal development in childhood hypothyroidism, and accelerated bone age and linear growth in childhood thyrotoxicosis **(Stevens DA *et al.*, *J. Bone Miner. Res.* 2000)**.

3. Thyroid hormones are essential for normal bone formation and mineralisation during skeletal development. *In vitro* studies in osteoblastic cell lines, bone marrow stromal cells and primary osteoblasts had indicated that T3 acts directly in bone-forming osteoblasts. Nevertheless, the mechanisms were poorly understood, downstream signalling responses to T3 had not been investigated and no T3 target genes had been identified. We sought to identify T3 target genes in osteoblasts using mRNA subtraction hybridisation and characterise downstream T3 responsive signalling pathways. Fibroblast growth factor receptor-1 (*Fgfr1*) was isolated from a pool of differentially expressed mRNAs and identified as the first T3-inducible target gene in osteoblasts. *Fgfr1* mRNA expression was stimulated 3-fold by T3 and FGFR1 protein was stimulated 4-fold. Induction of *Fgfr1* was independent of mRNA half-life and abolished by actinomycin D and cycloheximide, indicating that transcription and translation of a T3-responsive intermediary factor was required before *Fgfr1* expression increased. To determine whether the effects of T3 had functional consequences, we investigated the mitogen-activated protein kinase (MAPK) pathway and other second messenger systems activated by fibroblast growth factor 2 (FGF2) stimulation of FGFR1. T3 induced a more rapid response to FGF2 that was increased in magnitude by up to 3-fold, involved autophosphorylation of FGFR1 but not phosphorylation of the docking protein fibroblast growth factor receptor substrate-2 (FRS2), and was abolished by FGFR-selective inhibitors. By contrast, T3 did not influence the MAPK pathway in response to epidermal or platelet-derived growth factor stimulation and did not influence activation of the alternate FGFR1 signalling pathway via phospholipase Cγ. *In situ* hybridisation analysis of TRα-knockout mice, which have impaired bone formation and mineralisation, revealed decreased *Fgfr1* expression in osteoblasts and osteocytes. Furthermore, T3 failed to stimulate *Fgfr1* mRNA or enhance FGF2-activated MAPK signalling in TRα-null osteoblasts. The findings implicate FGFR1 signalling in the pathogenesis of skeletal disease caused by thyroid dysfunction. Thus, we identified a new pathway in osteoblasts in which T3 requires TRα to enhance FGF2-activated autophosphorylation of FGFR1 and downstream MAPK signalling. The studies suggest a novel pathogenic mechanism for skeletal diseases such as craniosynostosis resulting from childhood thyrotoxicosis **(Stevens DA *et al.*, *Mol. Endocrinol.* 2003)**.

4. Osteoporosis is characterised by low bone mass, deterioration of bone microarchitecture and increased susceptibility to fracture. The risk of fracture is determined by the peak bone mass acquired during growth and the rate of subsequent age-related bone loss. Our previous studies demonstrated essential roles for thyroid hormones in skeletal development, accrual of bone mass and bone mineralisation. Although thyrotoxicosis was well known to accelerate bone loss in adults, the role of thyroid hormones in bone maintenance and the pathogenesis of osteoporosis was unknown. Thus, it was unclear whether thyrotoxic bone loss results primarily from elevated thyroid hormones acting via TRs in the skeleton or whether it is due to deficiency of thyroid stimulating hormone (TSH) following suppression of the hypothalamic-pituitary-thyroid (HPT) axis. Regulation of thyroid status by the HPT axis feedback loop results in an inverse physiological relationship between thyroid hormone and TSH concentrations. Thus, it is challenging to discriminate the consequences of thyroid hormone excess from TSH deficiency *in vivo*. To clarify the roles of TRα, TRβ, and TSH in bone, we studied $TR\alpha^{0/0}$ and $TR\beta^{-/-}$ knockout mice, which lack all TRα or TRβ isoforms. $TR\alpha^{0/0}$ mice have normal levels of thyroid hormones and TSH, enabling the role of TRα to be determined directly. By contrast, thyroid hormones and TSH are both elevated in $TR\beta^{-/-}$ mice due to disruption of the HPT axis. Dissociation of the normal reciprocal relationship between thyroid hormones and TSH in $TR\beta^{-/-}$ mice enabled their relative contributions to bone maintenance and osteoporosis pathogenesis to be investigated. Euthyroid adult $TR\alpha^{0/0}$ mice displayed increased trabecular bone mass even though juveniles had growth retardation

with delayed endochondral ossification and reduced bone mineral deposition. By contrast, adult *TRβ*$^{-/-}$ mice, with elevated TSH and thyroid hormone levels, displayed an opposite phenotype consisting of osteoporosis despite the presence of advanced ossification and increased bone mineral in juveniles. Thus T3, acting via TRα, promotes anabolic effects during postnatal bone growth but has catabolic actions on the adult skeleton. Analysis of growth plate responses by *in situ* hybridisation demonstrated local growth hormone/insulin-like growth factor-1 signalling lies downstream of TRα in chondrocytes, while histomorphometry and back-scattered electron scanning electron microscopy (BSE-SEM) revealed TRα regulates osteoclastic bone resorption and bone turnover. Overall, impaired T3 action in the skeleton of *TRα*$^{0/0}$ mice, despite normal circulating thyroid hormone levels, is consistent with a primary role for TRα in skeletal development and bone maintenance. Osteoporosis with enhanced TRα-mediated T3 actions in the skeleton in *TRβ*$^{-/-}$ mice, in the presence of increased thyroid hormones and an elevated TSH concentration, is also consistent with a major role for thyroid hormones and TRα in the regulation of bone mass and the pathogenesis of osteoporosis **(Bassett JHD *et al.*, *Mol. Endocrinol.* 2007)**.

5. Clinical observations indicate that hypothyroidism in children delays ossification, bone mineralisation and linear growth whereas childhood thyrotoxicosis accelerates these processes, indicating that normal euthyroid status is essential for physiological development of the skeleton. In adults, persistent hypothyroidism results in decreased bone turnover with prolongation of the bone remodelling cycle and gradual accrual of bone mass and mineral over time. By contrast, thyrotoxicosis in adults causes high bone turnover osteoporosis with accelerated bone loss and an increased risk of fragility fracture. These clinical features are consistent with findings in *TRα*$^{0/0}$ and *TRβ*$^{-/-}$ knockout mice described above, which indicate T3 acts via TRα1 to mediate anabolic actions during skeletal growth but, paradoxically, induces catabolic responses in adult bone. We hypothesised that TRα-dependent bone growth and mineralisation during development is necessary to establish and maintain normal bone structure in adulthood. To determine how the effects of thyroid hormones during development manifest in adult bone, we studied *TRα1*$^{+/m}$ mice, which harbour a TRα1^{R384C} mutation that has decreased affinity for T3 and acts as a dominant negative antagonist of hormone bound TRs. Crucially, the dominant negative actions of TRα1^{R384C} can be overcome by increased concentrations of T3 *in vitro* and *in vivo* and we reasoned these mice could be used to determine the relationship between T3 actions during bone development and in adulthood. Adult *TRα1*$^{+/m}$ mice had osteosclerosis with increased bone mineralisation even though juveniles had delayed ossification. This phenotype was partially normalised by transient treatment of juveniles with T3 and fully reversed in *TRα1*$^{+/m}$*β*$^{-/-}$ double mutant mice due to 10-fold elevated hormone levels that allow the mutant TRα1 to bind T3. By contrast, deletion of TRβ in TRβ$^{-/-}$ mice, which causes a 3-fold increase of hormone levels, led to osteoporosis in adults but advanced ossification in juveniles. Target gene analysis revealed impaired thyroid signalling in *TRα1*$^{+/m}$ mice, increased signalling in *TRβ*$^{-/-}$ mice, and normal skeletal thyroid status in *TRα1*$^{+/m}$*β*$^{-/-}$ double mutants. Together, these studies show that TRα1 regulates both skeletal development and adult bone maintenance, with euthyroid status during development being essential to establish normal bone structure and mineralisation in adulthood. This study proved to be influential by proposing a unifying hypothesis that links T3-action during skeletal development to the consequences of thyroid hormone deficiency and excess in adult bone, a paradigm that has been extended to explain the developmental role of thyroid hormones in other tissues including the brain, gut and heart **(Bassett JHD *et al.*, *Mol. Endocrinol.* 2007)**.

6. Although the HPT axis efficiently controls circulating thyroid hormone and TSH concentrations within the physiological euthyroid range, it was recognised that circulating thyroid status does not necessarily correlate with thyroid status in T3 target tissues. The thyroid gland secretes the prohormone thyroxine (T4) and the active hormone 3,5,3'-L-triiodothyronine (T3). Most circulating T3 is derived from outer ring deiodination of T4 mediated by the type 1 iodothyronine deiodinase (DIO1). T4 and T3 enter target cells via active transport mediated by several membrane proteins, including the monocarboxylate transporters MCT8 and MCT10, organic anion transporter polypeptide 1c1 (OATPC1c1) and solute carrier family 17 member 4 (SLC17A4). The intra-cellular supply of T3 and its availability to the nuclear T3 receptor is controlled by the type 2 deiodinase (DIO2), which converts T4 to active T3 in target cells. In addition, a third enzyme (DIO3) inactivates T4 and T3 by inner ring deiodination to generate the inactive metabolites reverse T3 (3,3',5'-triiodothyronine, rT3) and T2 (3,3'-diiodothyronine) and

thus decrease TR-saturation. In general, the balanced activities of the DIO2 and DIO3 enzymes precisely determine the concentration of T3 available to the nuclear TR in target cells. Although the biochemical details of thyroid hormone metabolism were well known in 2008 the discovery of thyroid hormone transporters was new, and their physiological roles and mechanisms of action were incompletely understood. Importantly, the functional links between thyroid hormone uptake and metabolism in the regulation of T3-action in skeletal cells had not been explored. This study investigated whether the only known thyroid hormone transporter at the time, MCT8, is expressed in the skeleton and whether chondrocytes, osteoblasts and osteoclasts express functional deiodinases. Gene expression was analysed by RT-PCR and DIO1, DIO2 and DIO3 enzyme activities were measured by highly sensitive and specific assays that determined production of iodothyronine metabolites by cell lysates and intact cells using high performance liquid chromatography. MCT8 was expressed in chondrocytes, osteoblasts and osteoclasts at all stages of cell differentiation. DIO1 activity was undetectable in all skeletal cell types, DIO2 was only present in mature osteoblasts whereas DIO3 was evident throughout chondrocyte, osteoblast and osteoclast differentiation in primary cultures. These data demonstrate that activity of DIO2 is restricted but expression of MCT8 and DIO3 are widespread. Thus, T3 availability in bone may be limited mainly by DIO3-mediated catabolism, although restricted expression of DIO2 in osteoblasts suggests a role for T3 production in bone formation and mineralisation **(Williams AJ *et al.*, *Bone* 2008)**. In subsequent studies, we further demonstrated complex requirements for both MCT8 and MCT10 in the developing and adult skeleton *in vivo*, but no functional role for OATPC1c1 (Leitch *et al*., *Endocrinology* 2017, 158:3055-66; Lademann *et al*., *Endocrinology* 2022, 163(1) bqab218, https://doi.org/10.1210/endocr/bqab218).

7. The demonstration of restricted DIO2 activity in bone, led us to determine the role of *Dio2 in vivo*. We investigated *Dio2*$^{-/-}$ knockout and *Dio1*$^{-/-}$*Dio2*$^{-/-}$ double knockout mice, which both have normal circulating T3 concentrations. We hypothesised that *Dio2*$^{-/-}$ and *Dio1*$^{-/-}$*Dio2*$^{-/-}$ mice would display equivalent abnormalities of bone turnover and mineralisation that reflect the restricted activity of DIO2 in mature osteoblasts and the absence of DIO1 from all bone cell lineages. Bones from adult *Dio2*$^{-/-}$ mice were weak and brittle with reduced toughness, had a generalised increase in bone mineralisation and displayed increased susceptibility to fracture. Knockout mice had low bone turnover and impaired osteoblast activity with reduced mineralised surfaces and bone formation rate. Osteoclast function was unaffected, indicating Dio2$^{-/-}$ mice have an osteoblast-specific defect that results from local intracellular T3 deficiency despite the presence of a normal circulating concentration of T3. *Dio1*$^{-/-}$*Dio2*$^{-/-}$ double knockout mice had a similar phenotype to *Dio2*$^{-/-}$ mice, reflecting the absence of DIO1 activity in bone. Overall, brittle bone with reduced toughness and resistance to fracture in *Dio2*$^{-/-}$ and *Dio1*$^{-/-}$*Dio2*$^{-/-}$ mice reveals an essential role for DIO2 in osteoblasts in the physiological maintenance of bone mass and mineralisation, and in the optimisation of bone strength. The findings suggest a model in which restricted expression of DIO2 maintains a higher intracellular T3 concentration in osteoblasts relative to other skeletal cells that is essential for their normal function. As in other tissues, DIO2 activity in osteoblasts is upregulated in hypothyroidism and downregulated in hyperthyroidism, suggesting the enzyme acts as a local homeostatic regulator that buffers the detrimental effects of altered serum thyroid hormone levels on the skeleton. We proposed, therefore, that adverse effects of T3 deficiency on bone mineralisation are mitigated by increased DIO2-mediated conversion of T4 to T3 in osteoblasts, whereas inhibition of osteoblastic DIO2 activity limits the detrimental effects of thyroid hormone excess. Nevertheless, the capacity of this local feedback mechanism in osteoblasts is insufficient to compensate in overt hypothyroidism or thyrotoxicosis. Thus, optimal bone mineral content and resistance to fracture are maintained over the physiological range of systemic thyroid hormone concentrations by the regulated activity of DIO2 in osteoblasts. Absence of this compensatory mechanism in *Dio2*$^{-/-}$ mice results in cellular thyroid hormone deficiency and brittle bones. This seminal discovery demonstrated the fundamental physiological importance of cell-specific control of hormone metabolism in tissue homeostasis **(Bassett JHD *et al.*, *Proc. Natl. Acad. Sci. USA* 2010)**.

8. These studies in animal models convincingly demonstrate important roles for thyroid hormones in skeletal development and the regulation of bone mass and strength. They further identify physiological roles for T3 in the skeleton *in vivo* and uncover some of the molecular mechanisms of T3 action in bone and cartilage. Despite this, and the well-established association between untreated thyrotoxicosis and osteoporosis, the relationship between thyroid function and

bone mineral density (BMD) in humans remained unclear. Existing clinical studies were conflicted and confounded by differences in study design, inclusion of small numbers and heterogeneous groups of patients, and by sparse prospective data. We hypothesised that variation across the normal range of thyroid status in healthy postmenopausal women is associated with differences in BMD and fracture susceptibility. We studied a population-based cohort from five European cities comprising the Osteoporosis and Ultrasound Study (OPUS), a 6-year prospective study of fracture-related factors. A total of 2374 postmenopausal women participated, but individuals with thyroid disease and nonthyroidal illness and those receiving drugs affecting thyroid status or bone metabolism were excluded. Thus, a population of 1278 healthy euthyroid postmenopausal women was included, and we measured circulating free T4 (fT4), free T3 (fT3), TSH and bone turnover markers along with BMD and vertebral, hip and nonvertebral fractures. Higher fT4 and fT3 were associated with lower BMD at the hip, and higher fT4 was associated with increasing bone loss at the hip. After adjustment for age, body mass index and BMD, the risk of nonvertebral fracture was increased by 20% and 33% in women with higher fT4 or fT3, respectively. Higher TSH was protective and fracture risk was reduced by 35%. There were independent associations between fT3 and pulse rate, increased grip strength and better balance, indicating the relationship between thyroid status and fracture in humans is complex. This first prospective study of fracture-related factors in euthyroid post-menopausal women revealed that physiological variation in normal thyroid status is related to BMD and fracture risk **(Murphy E *et al.*, *J. Clin. Endocrinol. Metab.* 2010)**.

9. The studies in TR knockout and mutant mice described above, along with additional published work, established that thyroid hormones act via TRα in chondrocytes to regulate endochondral ossification and linear growth during skeletal development, and in osteoblasts to regulate adult bone mass and strength. It was not until 2012, however, that laboratories in Cambridge and Rotterdam independently identified the first children with mutations in the *THRA* gene encoding TRα. Affected individuals had a bone dysplasia with short stature that was similar to the skeletal features resulting from congenital hypothyroidism, and which recapitulated the abnormal phenotypes we had characterised in mice with *Thra* mutations. In the context of studies in TR knockout and mutant mice, the findings in patients with *THRA* mutations confirm that thyroid hormones and TRα have essential roles in the skeleton that are conserved between species. We hypothesised that $TR\alpha1^{PV/+}$ mice, which express a potent dominant-negative mutant TRα1 similar to affected patients with *THRA* mutations, would represent an excellent disease model to (i) predict the skeletal outcome in individuals with *THRA* mutations and (ii) investigate whether prolonged treatment with a supraphysiological dose of T4 could ameliorate the skeletal abnormalities. $TR\alpha1^{PV/+}$ mice had short stature, grossly abnormal bone morphology and mineralisation with decreased osteoclastic bone resorption and high bone mass but normal bone strength. Although T4 treatment suppressed TSH secretion, it had no effect on skeletal maturation, linear growth, or bone mineralisation, thus demonstrating profound cellular resistance to thyroid hormone in bone. Despite this, prolonged T4 treatment abnormally increased bone stiffness and strength, suggesting the potential for detrimental consequences of treatment with supraphysiological doses of T4 in the long term. Together with studies described above in $TR\alpha1^{+/m}$ mice, which express a milder mutation ($TR\alpha1^{R384C}$), the findings in T4 treated $TR\alpha1^{PV/+}$ mice predicted correctly that patients with different *THRA* mutations would display variable responses to T4 treatment that depend on the severity of the causative mutation and the resulting dominant-negative properties of the mutant TRα1 **(Bassett JHD *et al.*, *Endocrinology* 2014)**.

10. Our findings showing that variation in normal thyroid status is related to fracture risk led to considerable international interest. The OPUS study was invited to join the Thyroid Studies Collaboration (TSC) (https://www.thyroid-studies.org) and I joined the TSC Steering Committee. The TSC was formed in 2007 and now comprises pooled data from more than 100,000 participants from 25 cohorts in Europe, Asia, USA and South America. It represents the largest worldwide collaboration into thyroid epidemiology and focuses studies into (i) outcomes related to thyroid dysfunction and (ii) risk factors for thyroid dysfunction. Further to the findings in OPUS, we joined the TSC to investigate whether subtle alterations in thyroid function that occur in subclinical thyroid dysfunction, defined as abnormal TSH with normal fT4, were associated with bone loss and increased fracture risk. Prospective studies were in conflict at the time because of heterogeneity and inclusion of small numbers of individuals with subclinical dysfunction who incurred low numbers of incident fracture events. We performed a pooled analysis of individual

participant data from 13 prospective cohorts comprising 70,298 participants during more than 750,000 person-years of follow-up that assessed the association of subclinical thyroid dysfunction with risk for hip, non-spine and spine fractures as well as fractures at any location. This approach allowed exploration of the relationship of age, sex, and TSH levels with the association between subclinical thyroid dysfunction and fractures. Subclinical hyperthyroidism in both men and women was associated with a greater risk of hip (36% increased risk) and other fractures (28% for any fracture; 16% for non-spine; 51% for spine), particularly among those with TSH levels suppressed lower than 0.10 mIU/L (61% increase for hip, 98% for any fracture; 61% for non-spine; 357% for spine) and those with endogenous subclinical hyperthyroidism (52% increase for hip, 42% for any fracture; 74% for spine). There were no associations between subclinical hypothyroidism and fracture risk. Overall, this seminal paper provided conclusive evidence that subclinical hyperthyroidism is associated with an increased risk of fracture **(Blum M *et al*., *JAMA* 2015)**.

Summary: Contributions to Field of Thyroid Hormones and Bone

These contributions identified the fundamental role of thyroid hormones during bone development and maintenance, revealing the cellular and molecular mechanisms that underpin the relationship between thyroid status and skeletal health. I cloned two TR isoforms, characterised the major mechanisms of T3 action in the skeleton, identified key T3 target genes in bone and cartilage, discovered key roles for the T3-transporters MCT8 and MCT10 during skeletal development and in adult bone, and showed that generation of T3 in osteoblasts by the DIO2 enzyme is essential to optimise adult bone structure, mineralisation and strength. This body of work unified the anabolic actions of T3 during skeletal development with catabolic effects in adult bone and showed that T3 responses are mediated by a canonical signalling pathway that requires binding of TRα to DNA. Physiological studies established the paradigm that T3-target tissues are primarily responsive to either TRα or TRβ *in vivo* and predicted the skeletal dysplasia and therapeutic response in patients recently characterised with *THRA* mutations. Subsequent prospective studies and three large individual participant meta-analyses showed that variations in thyroid status are related to bone loss, BMD and fracture susceptibility. These discoveries changed clinical practice by underpinning the latest international guidelines that I co-authored, which (i) focus long-term management to limit the detrimental skeletal consequences of thyroid hormone excess in thyroid cancer survivors (Perros et al., *Clin. Endocrinol.* 2014, 81:1-122), and (ii) have changed management goals in the treatment of patients with hypothyroidism (Okosieme et al., *Clin. Endocrinol.* 2016, 84:799-808). This body of work resulted in invitations to contribute the definitive review of the field (Bassett & Williams, *Endocr. Rev.* 2016, 37(2):135-187) and write original chapters in major international textbooks, including *Harrison's Principles of Internal Medicine* (2002), *Oxford Textbook of Endocrinology* (2002, 2011 & 2019) and *Werner & Ingbar's The Thyroid: A Fundamental and Clinical Text* (2012 & 2020). I served as President of the British Thyroid Association (2011-2014), the Society for Endocrinology (2016-2019) and the European Thyroid Association (2021-2023), and was an expert advisor to the Scottish Parliament Public Petitions Committee (2013) on the treatment of hypothyroidism and to the Governments of Kuwait (2010) and United Arab Emirates (2013) on thyroid clinical service provision. My work was recognised by the Society for Endocrinology Medal (2011), Bermuda Hospitals Board Edwin B. Astwood Memorial Prize (2011), European Thyroid Association Merck Serono Prize (2012), Danish Thyroid Association Distinguished Annual Lectureship (2014), American Thyroid Association Sidney H. Ingbar Award (2014) and British Thyroid Association Rosalind Pitt-Rivers Medal (2016).

B) Pathophysiology of Osteoporosis and Osteoarthritis

While working on the role of thyroid hormones in bone, we secured funding from the Wellcome Trust (2012-2022) to obtain the necessary laboratory infrastructure and develop state-of-the-art expertise in skeletal biology for investigation and functional annotation of novel genes associated with osteoporosis and osteoarthritis. Alongside existing light and con-focal microscopy capability, we acquired Faxitron MX20 and UltraFocus digital X-ray microradiography systems; a Scanco µCT-50 system for micro-computerised tomography (µCT); a bespoke Tescan Vega3 XMU scanning electron microscope with Deben four-quadrant back-scattered electron detector for BSE-SEM; and an Instron 5543 load frame for biomechanical testing. We established collaborations with the International Mouse Phenotyping Consortium (IMPC), major international Genome-Wide Association Study (GWAS) consortia in osteoporosis and osteoarthritis, and

leading experts in genetics, bioinformatics and “big-data” integration and analysis.

11. Osteoporosis is the commonest skeletal disorder affecting hundreds of millions of people worldwide and costing tens of billions of pounds each year. Between 50 and 85% of the variance in bone mineral density (BMD) is genetically determined but only a fraction is accounted for by known genetic variation, and the vast majority of genes involved remain to be identified. We developed and validated a rapid-throughput multi-parameter skeletal phenotype screen to identify functionally significant skeletal phenotypes in knockout mice generated by the Wellcome Trust Sanger Institute (WTSI) Mouse Genetics Project (MGP) in order to discover novel genes involved in the pathogenesis of osteoporosis. We integrated quantitative X-ray microradiography, µCT and biomechanical testing data with bone densitometry data from 100 unselected knockout strains generated by the IMPC and used Mahalanobis distance calculation and principal component analysis to analyse the data. We identified nine genes that determine bone mass and strength, including five (*Bbx, Cadm1, Fam73B, Prpsap2, Slc38a10)* whose deletion results in low bone mass and four *(Asxl1, Setdb1, Spns2, Trim45*) whose deletion results in high bone mass. None had been implicated previously in skeletal disorders and analysis of the biomechanical consequences of their deletion identified a new functional classification that relates bone structure to bone strength. Bones from *Bbx, Cadm1* and *Fam73b* knockout mice were weak but flexible with reduced maximum load and they retained the capability to bend and dissipate energy prior to fracture. *Prpsap2* and *Slc38a10* bones were weak and brittle with decreased maximum load but lacked the capability to dissipate energy prior to fracture. *Asxl1, Trim45, Spns2* and *Setdb1* bones were strong but brittle with an increased maximum load and were unable to dissipate energy prior to fracture. Overall, bones from wild-type mice were strong and flexible with normal bone mineral content (BMC), whereas knockout mouse bones were either (i) weak but flexible with low BMC, (ii) weak and brittle with low BMC or (iii) strong but brittle with high BMC. Thus. we established a new rapid-throughput phenotyping strategy to identify a functional classification of bone structure and strength that provides original insights into skeletal biology and new opportunities for gene discovery and functional annotation of candidate loci associated with osteoporosis and other skeletal disorders **(Bassett JHD *et al., PLoS Genet.* 2012)**.

12. To investigate whether phenotyping knockout mice with abnormal bone mass and strength could be used to infer causation for genes associated with osteoporosis in humans, we developed collaborations with the GEnetic Factors for OSteoporosis (GEFOS) Consortium (http://www.gefos.org) and geneticists in Europe, North America and Australia. In previous osteoporosis GWAS, BMD data were derived from dual-energy X-ray absorptiometry (DXA), which is expensive. DXA GWAS were limited to a population of up to 32,965 individuals in the largest study at the time, which compromised the ability to detect risk loci sensitively and robustly. An alternative method of determining BMD that is quick, safe and inexpensive uses ultrasound at the heel, referred to as estimated BMD (eBMD). Ultrasound-derived eBMD is highly heritable, independently associated with fracture risk and can be used in very large cohorts. We undertook a GWAS of 142,487 individuals from the UK Biobank (https://www.ukbiobank.ac.uk) to determine eBMD-associated loci. We identified 307 conditionally independent single-nucleotide polymorphisms (SNPs) that attained genome-wide significance at 203 loci, explaining 12% of the phenotypic variance. The findings confirmed the nine loci identified in a previous eBMD GWAS as well as 55 of 73 loci previously associated with BMD at other skeletal sites using DXA. Thus, we identified 153 previously unreported loci, and several rare variants with large effect sizes. To prioritise candidate genes and investigate underlying mechanisms, we undertook (i) bioinformatic functional genomic annotation and human osteoblast expression studies; (ii) gene-function prediction; (iii) skeletal phenotyping of 120 genes in IMPC knockout mice with deletions of genes adjacent to lead independent SNPs; and (iv) analysis of gene expression in mouse osteoblasts, osteocytes and osteoclasts. Forty-three IMPC knockout mouse lines had significantly abnormal skeletal phenotypes, including mice with deletion of *Gpc6* and other genes encoding heparan sulfate core proteins, synthetic and modifying enzymes. Overall, the study provided important new insights into pathophysiological mechanisms that underlie changes in BMD and fracture risk in humans. The prioritised genes identify signaling pathways that represent novel drug targets for the prevention and treatment of osteoporosis **(Kemp JP *et al., Nat. Genet.* 2017)**.

13. A major challenge in the analysis of complex diseases concerns how to map associated loci to causal genes. Highly polygenic traits such as BMD, a strong predictor of fracture risk, allow

empirical testing of which methods can be used to best link associated SNPs to disease causation. Known causative genes and their encoded proteins involved in the pathogenesis of osteoporosis can be identified when their therapeutic manipulation results in changes in BMD or when mutations with Mendelian inheritance segregate with skeletal disease in affected families. Given a sufficient number of loci identified in large-scale GWAS, we hypothesised that (i) different genomic characteristics linking associated SNPs to known causal genes (e.g. cell-specific 3-dimensional contact domains and open chromatin states, physical proximity and the presence of associated coding variants) could be identified, (ii) rapid-throughput phenotyping of knockout mice generated by large-scale studies could be used to identify genes whose deletion results in an abnormal skeletal phenotype, and (iii) GWAS data, genomic characteristics and mouse phenotype data could be integrated to generate a comprehensive functional genetic atlas that identifies novel causative genes and describes the genetic and molecular pathogenesis of osteoporosis. We undertook an eBMD GWAS of 426,824 individuals in the UK Biobank, identifying 301 novel loci, which explain 20% of its variance, and identified genetic determinants of fracture in 1.2 million individuals by combining the UK Biobank and 23andMe cohorts. We then assessed SNP-level and genomic landscape characteristics, mapping associated SNPs to genes known to influence bone density and strength. Target genes identified in this way were enriched 58-fold for known causal genes and for genes differentially expressed in osteocytes *in vivo*. We next performed rapid-throughput skeletal phenotyping of 126 IMPC knockout mice with disruptions in predicted target genes and found a significantly increased abnormal skeletal phenotype frequency (3.2-fold enrichment, $P<0.0001$) compared to 526 unselected KO mouse lines. Convergence of evidence from human and mouse genetics, bone cell expression, and cell culture experiments pointed to a role for *DAAM2* in osteoporosis. *Daam2*$^{-/-}$ mice had a disproportionate decrease in bone strength relative to mineralisation with increased cortical bone porosity, suggesting a role for non-canonical Wnt signalling in the determination of bone quality. The genetic atlas defined by this work provides evidence linking associated SNPs to causal genes, identifies 301 novel eBMD loci and offers new insight into osteoporosis pathophysiology, highlighting opportunities for biomarker discovery and drug development **(Morris JA *et al., Nat. Genet.* 2019)**.

14. One such example relates to iron homeostasis. Systemic Fe concentrations are inversely related to BMD and regulated by optimising Fe absorption from the gut, limiting Fe export into the circulation and recycling Fe from degraded red blood cells. Abnormalities in this system result in Fe overload, and mutations in key regulatory genes including *HFE* (encoding homeostatic Fe regulator), *HJV* (hemojuvelin), *HAMP* (hepcidin) and *TFR2* (transferrin receptor 2) cause hereditary haemochromatosis with premature osteoporosis. Although the relationship between Fe overload and early onset osteoporosis was well established, the underlying mechanisms and role of Fe homeostasis in the regulation of bone turnover were unknown. TFR2 is critical, controlling Fe uptake and degradation in the liver by HAMP, its efflux from the spleen, and sensing the circulating transferrin-bound Fe concentration. TFR2 acts via bone morphogenetic protein (BMP) downstream signalling, and inhibition of this pathway results in Fe overload. Given the key role of BMPs in the regulation of bone turnover, we hypothesised that, in addition to its role in Fe homeostasis, TFR2 acts via BMP signalling in the skeleton to maintain bone mass and strength. We studied *Tfr2*$^{-/-}$ knockout mice and *Tfr2* conditional knockout mice lacking *Tfr2* in hepatocytes (crossing *Tfr2* floxed mice with *Alb-cre* mice), osteoblasts (*Osx-cre*) or early (*Lysm-Cre*) and mature (*Ctsk-cre*) osteoclasts. We performed dietary manipulation, Fe chelation studies, ovariectomy and bone marrow transplantation experiments. Mice lacking *Tfr2* had increased bone mass and mineralisation independent of Fe homeostasis. Bone marrow transplantation studies and analysis of cell-specific *Tfr2* knockout mice revealed that TFR2 impairs BMP-p38MAPK signalling and decreases expression of sclerostin, a Wnt inhibitor, specifically in osteoblasts. Accordingly, reactivation of MAPK or overexpression of sclerostin rescued skeletal abnormalities in *Tfr2*$^{-/-}$ mice. We further showed the extracellular domain of TFR2 binds BMPs and inhibits BMP2-induced heterotopic ossification by acting as a decoy receptor. Thus, TFR2 limits bone formation by modulating BMP signalling and inducing expression of the Wnt inhibitor sclerostin. TFR2 interacts directly with BMP either as a receptor or as a co-receptor in complex with other BMP receptors. These studies identify *Tfr2* as a novel regulator of bone homeostasis that inhibits osteoblastic bone formation and reveal mechanisms by which Fe homeostatic pathways regulate bone turnover **(Rauner M *et al., Nat. Metab.* 2019)**.

15. Bone mineralisation is a tightly regulated process involving progressive mineralisation of

collagen fibrils by osteoblasts and requiring sufficient concentrations of calcium and inorganic phosphate. The phosphate transporters *SLC20A1* and *SLC20A2* are expressed in bone cells along with *NPT2A*, which is also found in the proximal tubule where it regulates renal phosphate reabsorption. *SLC20A1* and *SLC20A2* were traditionally thought to have major roles in skeletal mineralisation but studies in *Slc20a1*$^{-/-}$ mice did not support this view. However, the identification of *SLC20A2* mutations in patients with autosomal dominant familial brain calcification and idiopathic basal ganglia calcification suggested a key role for *SLC20A2* in the regulation of tissue mineralisation. *Slc20a2*$^{-/-}$ mice recapitulate the human brain calcification syndromes but were also found in preliminary analyses to have short stature, dental abnormalities, cataracts and deviation of the nasal bones. These findings suggested a more general role for *SLC20A2* in tissue mineralisation and, in view of its expression in osteoblasts, we hypothesised *SLC20A2* has an important, but unknown, physiological role in the skeleton. Juvenile *Slc20a2*$^{-/-}$ mice had abnormal endochondral and intramembranous ossification, decreased mineral accrual, abnormal tooth development and mineralisation, and short stature. Adult *Slc20a2*$^{-/-}$ mice had only small decreases in bone mass and mineralisation but profoundly impaired bone strength. In biomechanical testing studies the yield load, maximum load, and stiffness of femurs were all markedly below values predicted from their bone mineral content as determined in a cohort of 320 wild-type control mice from an identical genetic background. Bone quality is the term used to describe properties of bone composition and structure that contribute to strength independently of mineral content and it has emerged as an important and enigmatic priority in osteoporosis research. These studies identify *Slc20a2* as a physiological regulator of tissue mineralisation in various organs and highlight its critical role in the determination of bone quality. Preliminary analysis of µCT imaging of the skull in a cohort of patients with familial brain calcification identified abnormalities of cranial bone density and thickness, suggesting such patients should also be investigated for additional skeletal and dental abnormalities. Interestingly, *SLC20A1* was recently identified by GWAS as a candidate gene for clinical vertebral fractures independent of bone density, further suggesting phosphate transport is an essential determinant of bone quality **(Beck-Cormier S *et al., J. Bone Miner. Res.* 2019)**.

16. We previously investigated genetic determinants of cell multinucleation using inbred Wistar Kyoto (WKY) rats that display spontaneous macrophage fusion and identified a co-expression network (MMnet) enriched for genes expressed in osteoclasts (Kang et al., *Cell Rep.* 2014, 8(4):1210-1214). Functional characterisation of cell-specific regulatory networks is a powerful way to establish causal links between genetic variation and phenotype. The osteoclast offers a rare opportunity to investigate the contribution of co-regulated genes to *in vivo* phenotype as its multinucleation and resorption activities *in vitro* correlate well with quantifiable skeletal traits. We hypothesised that functional analysis of MMnet genes *in vitro* and in knockout mice would identify genes that control osteoclast multinucleation and hydroxyapatite resorption *in vitro* and also regulate bone structure and strength *in vivo*. We showed that bone marrow precursors from WKY rats display increased osteoclastogenesis *in vitro* compared to Lewis (LEW) rats in which spontaneous macrophage fusion does not occur. In addition, WKY rats had markedly decreased bone strength resulting from low trabecular bone volume and mineral content and decreased cortical bone thickness and BMD. Together, these data suggested that MMnet regulates adult bone homeostasis via its actions in osteoclasts. We next showed that MMnet genes were enriched for skeletal disease candidate genes identified in GWAS, while mutations affecting nine MMnet genes cause rare monogenic skeletal disorders. We investigated IMPC knockout mice with deletions of twelve MMnet genes and six of these (*Appl2*$^{-/-}$, *Atp8b2*$^{+/-}$, *Deptor*$^{-/-}$, *Eml1*$^{-/-}$, *Igsf8*$^{-/-}$, *Pik3cb*$^{-/-}$ mice) had skeletal abnormalities, with decreased bone strength in *Atp8b2*$^{+/-}$, *Deptor*$^{-/-}$ and *Eml1*$^{-/-}$ mice. siRNA knockdown studies in human osteoclast precursors and analysis of knockout mice demonstrated an inverse relationship between effects on osteoclastogenesis *in vitro* and bone mass *in vivo*. Furthermore, conditional deletion of the MMnet gene *Slc40a1* in osteoclasts resulted in increased bone mass and strength due to impaired osteoclast multinucleation and function. These studies pinpoint MMnet as a functionally conserved network that regulates osteoclast multinucleation and bone mass and identify new genetic determinants of bone mass and strength that act in osteoclasts to regulate skeletal homeostasis **(Pereira M et al., *eLife* 2020)**.

17. Osteoclasts participate in the bone remodelling cycle with osteoblasts to maintain bone mass. The activities of osteoclasts and osteoblasts are coordinated and coupled in both time and

space throughout the skeleton. Enhanced osteoclastic bone resorption, decreased osteoblastic bone formation, or a combination of both occurs in osteoporosis and results in bone loss, leading to an increased risk of fracture. Impaired osteoclast function, by contrast, shifts the balance of the bone remodelling cycle towards a net gain in osteoblast activity with accumulation of bone mass and mineralisation, as seen in osteopetrosis. Osteoclasts are unique, large multinucleated cells with the ability to remove old bone: they are formed by fusion of monocyte/macrophage-derived precursor cells and are thought to undergo apoptosis once bone resorption has been completed. Using intravital imaging, we showed that receptor activator of nuclear factor kappa-B ligand (RANKL) stimulated osteoclasts undergo a novel fate of fission into daughter cells called "osteomorphs", which then fuse to reform osteoclasts. This cellular recycling enables large multinucleated osteoclasts to be broken down into smaller osteomorphs that can migrate via the bone marrow or in the circulation, enabling fusion and regeneration of multinucleated osteoclasts at new resorption sites elsewhere in the skeleton. By contrast, blockade of RANKL signalling inhibits cellular recycling resulting in accumulation of osteomorphs. Single-cell RNA sequencing revealed that osteomorphs are distinct from osteoclasts and macrophages. They express non-canonical osteoclast genes associated with abnormal structural and functional skeletal phenotypes when deleted in mice. Furthermore, genetic variation in human orthologues of osteomorph genes associates with BMD, while mutations in several osteomorph genes cause monogenic skeletal disorders. In summary, these studies identified a new cell type, the osteomorph, and uncovered an unexpected new cellular programme for the reuse of osteoclasts that involves their dissolution, transport and reassembly at distant sites. The findings overturn the long-standing premise that osteoclasts differentiate solely from hematopoietic precursors and undergo apoptosis after completing resorption. Studies in knockout mice revealed that genes with enriched expression in osteomorphs determine bone structure and function, while analysis of human genetic data and monogenic disease databases demonstrated that osteomorph genes are implicated in inherited skeletal disorders **(McDonald MM *et al., Cell* 2021)**.

18. The skeleton is highly dynamic structure, continuously modelling and remodelling its shape and mineral content. Osteocytes are master regulators of the skeleton, derived from terminally differentiated osteoblasts. They are the most abundant cell type in bone but have been difficult to study because of their location within mineralised matrix. Osteocytes are connected via a network of dendritic processes distributed throughout mineralised bone. The scale and complexity of the network is comparable to neurons in the brain, with 42 billion osteocytes present in the human skeleton estimated to form 23 trillion connections. This network enables osteocytes to detect and respond to mechanical strain, hormones and local growth factors and cytokines. Osteocytes react to these signals by regulating and coordinating the formation and activity of osteoclasts and osteoblasts, instructing these cells to repair damaged bone, controlling bone mass and composition, and ensuring the optimal distribution of bone tissue in response to mechanical stress and injury. Osteocytes also remove and replace bone surrounding the osteocyte network by a process known as peri-lacunar remodelling, thus liberating calcium and phosphate in response to endocrine demands. Osteocytes have additional regulatory roles beyond the skeleton, including in skeletal muscle, adipose tissue, the central nervous system and in the control of phosphate homeostasis and energy expenditure, indicating the network also acts as an important endocrine organ. We developed novel methods to remove bone marrow, skeletal muscle, adipocytes and growth plates from long bones and efficiently isolate osteocytes for RNAseq analysis. We mapped the osteocyte transcriptome from different skeletal sites (femur, tibia, humerus) in male and female mice at several ages in order to reveal the genes and molecular programs that control this complex cellular network. Gene expression analysis revealed bone-specific and sexually dimorphic differences in the osteocyte transcriptome during post-natal development as well as lactation-specific differences in post-partum females. We defined an osteocyte transcriptome signature of 1239 genes that distinguishes osteocytes from other cell types. Seventy-seven percent of signature genes have no previously known role in the skeleton, and they are enriched for genes that regulate neural network formation, suggesting repurposing of this programme to play an important role in osteocyte network communication. To establish whether osteocyte transcriptome signature genes also have a functional role in the skeleton, we extended our rapid throughput phenotype analysis of IMPC knockout mice to include 19 parameters relating to trabecular and cortical bone structure and strength. Skeletal phenotyping of 733 unselected knockout mouse lines, of which 64 had deletions of genes present in the osteocyte signature, resulted in identification of 26 osteocyte transcriptome signature genes

that control bone structure and function, of which 15 were not known to have a role in the skeleton. We further showed that osteocyte transcriptome signature genes are enriched for human orthologs that cause monogenic skeletal disorders or are associated with osteoporosis and osteoarthritis candidate genes identified in large scale GWAS. These studies reveal the molecular landscape that regulates osteocyte network formation and function and establish the importance of osteocytes in human skeletal disease **(Youlten SE *et al*., *Nat. Commun.* 2021).**

19. Osteoarthritis is the commonest joint disease afflicting over 500 million people worldwide. It is a severe, debilitating and chronic disease affecting all tissues in the joint that is characterised by cartilage degeneration and synovial hypertrophy. The lifetime risk of developing symptomatic disease is estimated between 25-45% and its incidence is rising in an increasingly obese and ageing population. Risk factors for osteoarthritis include older age, female sex, obesity, joint morphology, injury, and family history. The heritability of osteoarthritis has been estimated to range between 40-60%, and GWAS have identified around 100 risk loci that replicate robustly in additional populations. Nevertheless, the molecular mechanisms of disease pathogenesis have not been elucidated and genes that are specifically expressed in diseased joint tissues have not been determined. Currently, the only treatment for patients with osteoarthritis is pain relief or joint replacement surgery, highlighting the need for improved understanding of its pathophysiology. We collected low-grade visually intact and high-grade severely damaged cartilage and synovial tissue from 115 patients undergoing joint replacement for osteoarthritis. Availability of paired preserved and severely damaged joint tissue samples from the same individual enables the two disease states to be compared in affected primary tissues. RNA extracted from cartilage and synovium was profiled by RNAseq, and cartilage samples were additionally investigated using quantitative proteomics. We also generated genome-wide genotype data from peripheral blood. By integrating genotype data with transcriptomic and proteomic datasets we characterised the molecular features of cartilage degradation, creating a gene expression profile of joint degeneration and identifying (i) genetic variants influencing mRNA or protein levels in molecular quantitative trait loci (molQTLs) for each tissue type, (ii) likely functional effector genes for osteoarthritis-associated GWAS signals (including *NPC1, FAM53A, SMAD3, SLC44A2, ALDH1A2*), and (iii) tractable potential therapeutic targets for drug development and repurposing. The benefit of integrating large multi-omic datasets with GWAS data was evidenced by identification of an association between 91 established osteoarthritis variants with disease state, cell-specific gene expression and proteomic profiles. This study emphasises the need for multidisciplinary research to progress understanding of the pathogenesis underlying complex chronic diseases such as osteoarthritis **(Steinberg J *et al*., *Nat. Commun.* 2021).**

20. Osteoarthritis causes articular cartilage damage and loss together with structural abnormalities of subchondral bone and low-grade joint inflammation. To identify new drug targets for osteoarthritis, it is essential to establish where the initiating events occur in disease onset, how communications between cartilage, bone and synovial cells influence disease progression, and what mechanisms underlie disease pathogenesis. Human studies are complicated by inclusion of patients of differing age, sex, ethnicity and aetiology. They lack critical comparative information from normal joints as only tissue obtained at joint replacement for end-stage disease can be investigated. Thus, human studies alone cannot establish causal relationships that connect changes in gene expression with mechanisms of disease. A new approach that uses a well-established disease model is essential to advance the field. We hypothesised that developing and validating a disease model in the mouse would accelerate gene discovery in osteoarthritis, increase our understanding of joint physiology and disease pathogenesis, and facilitate identification of drug targets that prevent or delay joint destruction. We invented and applied rapid-throughput phenotyping methods, which uniquely quantify key abnormalities of osteoarthritis in the mouse knee in three dimensions. Cartilage damage is a cardinal feature, and we developed a non-destructive method for joint surface imaging using BSE-SEM. The knee joint surface is moulded in its native hydrated state using high-resolution silicon, an acrylic cast is imaged, and articular cartilage damage quantified. Loss of cartilage volume is pathognomonic during osteoarthritis progression, and we developed iodine contrast-enhanced µCT (ICE-µCT) to enable simultaneous three-dimensional segmentation of articular cartilage and subchondral bone. Subchondral bone abnormalities often accompany cartilage damage, and we demonstrated the sensitivity of X-ray microradiography for high-throughput determination of subchondral bone mineral content. These methods were validated using (i) the gold standard surgical provocation

model, destabilisation of the medial meniscus (DMM), (ii) the Osteoarthritis Research Society International (OARSI) histological scoring system, and (iii) osteophyte and synovitis outcome scores. We applied the methods to phenotype knee joints from 50 randomly selected IMPC knockout mouse lines and identified seven with grossly abnormal phenotypes, including mice haplo-insufficient for the homeobox gene *Pitx1*, which was found to have a protective function. Prioritisation using statistical and bioinformatic analysis of gene expression, human monogenic disease, GWAS and PubMed databases highlighted a further seven knockout lines, resulting in identification of 14 genes with evidence for a functional role in osteoarthritis pathogenesis. We next interrogated our database of joint phenotypes from knockout mice with a list of 409 genes differentially expressed in preserved versus severely damaged human osteoarthritis cartilage. This analysis identified six genes (*UNK, JOSD1, GSDME, ARHGAP30, CCDC6, COL4A2*) that caused abnormal joint phenotypes when deleted in knockout mice. These genes had not previously been linked to osteoarthritis in GWAS, demonstrating the synergy and utility of combining mouse and human approaches for discovery of novel osteoarthritis susceptibility genes. We next applied rapid-throughput phenotyping to characterise early features of age-related joint degeneration in 1-year old mice and demonstrated its sensitivity to detect disease onset as well as surgically provoked late-stage disease, paving the way for application to analysis of drug intervention studies. Finally, we phenotyped CRISPR/Cas9 mutant mice with a Thr92Ala polymorphism in the *Dio2* gene that is orthologous to a human variant associated with osteoarthritis and demonstrated this allele confers protection against early onset disease. Overall, our new rapid-throughput joint phenotyping methods and expanding resource of mutant mice will accelerate functional gene discovery in osteoarthritis and offer drug discovery opportunities for this incapacitating disease **(Butterfield NC *et al*., *Nat. Commun.* 2021).**

Summary: Contributions to Field of Pathophysiology of Osteoporosis and Osteoarthritis

These studies have resulted in substantial advances in the field of osteoporosis and osteoarthritis research. Crucial contributions to pivotal osteoporosis GWAS identified 518 bone mineral density and 13 fracture loci that now account for over 20% of the population variance in bone density. We identified over 300 novel eBMD loci and validated numerous genes, including (i) transferrin receptor-2 as a novel regulator of bone mass acting via BMP/p38MAPK/Wnt signalling, (ii) over 100 genes that determine bone mass, quality and strength comprising enzymes, ion and amino acid transporters, cell cycle regulators, transcription factors and modulators of non-canonical Wnt signalling, and (iii) age-specific and sexually dimorphic genetic effects on bone mineral density. Recent discoveries include (i) identification of a new cell type with a unique transcriptome, termed the “osteomorph”. Bone resorbing multinucleated osteoclasts undergo cycles of cell fission and fusion, recycling via osteomorphs in the bone marrow to regulate osteoclast motility and dynamic bone remodelling *in vivo*, (ii) elucidation of a transcriptome map of genes expressed in osteocytes, the master regulatory cells in bone. Osteocyte signature genes correlate closely with loci identified in human GWAS and in the nosology of monogenic skeletal disorders, establishing the cellular pathogenesis of various skeletal diseases, and (iii) development of novel imaging methods in osteoarthritis disease models and generation of the first multi ‘omic molecular QTL map of human disease to accelerate causative gene discovery in osteoarthritis. This multidisciplinary and international approach is transformative and has resulted in a comprehensive atlas of human and murine genetic influences on bone and joint disease that offer novel insights into the pathophysiology of osteoporosis and osteoarthritis with exciting opportunities for biomarker discovery and drug development. This body of work has resulted in invitations to contribute seminal chapters in major international textbooks, including (i) *Genetics of Bone Biology and Skeletal Disease* (2018) and (ii) *Osteoporosis* (2020), and Plenary Lectures to the (i) 21st World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (IOF-ESCEO WCO), (ii) ASBMR Bone Turnover Markers Annual Meeting, and (iii) 9th Congress of the Romanian Society of Osteoporosis and Musculoskeletal Diseases (all in 2021). My work was recognised by the European Calcified Tissue Society Steven Boonen Clinical Research Award (2018), and I was elected Fellow of the Academy of Medical Sciences (2019), Member of Academia Europaea (2021) and Fellow of the Association of Physicians of Great Britain & Ireland (2021).