REVIEW

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Early-Onset Osteoporosis: Rare Monogenic Forms Elucidate the Complexity of Disease Pathogenesis Beyond Type I Collagen

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ABSTRACT

Early-onset osteoporosis (EOOP), characterized by low bone mineral density (BMD) and fractures, affects children, premenopausal women and men aged <50 years. EOOP may be secondary to a chronic illness, long-term medication, nutritional deficiencies, etc. If no such cause is identified, EOOP is regarded primary and may then be related to rare variants in genes playing a pivotal role in bone homeostasis. If the cause remains unknown, EOOP is considered idiopathic. The scope of this review is to guide through clinical and genetic diagnostics of EOOP, summarize the present knowledge on rare monogenic forms of EOOP, and describe how analysis of bone biopsy samples can lead to a better understanding of the disease pathogenesis. The diagnostic pathway of EOOP is often complicated and extensive assessments may be needed to reliably exclude secondary causes. Due to the genetic heterogeneity and overlapping features in the various genetic forms of EOOP and other bone fragility disorders, the genetic diagnosis usually requires the use of next-generation sequencing to investigate several genes simultaneously. Recent discoveries have elucidated the complexity of disease pathogenesis both regarding genetic architecture and bone tissue-level pathology. Two rare monogenic forms of EOOP are due to defects in genes partaking in the canonical WNT pathway: *LRP5* and *WNT1*. Variants in the genes encoding plastin-3 (*PLS3*) and sphingomyelin synthase 2 (*SGMS2*) have also been found in children and young adults with skeletal fragility. The molecular mechanisms leading from gene defects to clinical manifestations are often not fully understood. Detailed analysis of patient-derived transiliac bone biopsies gives valuable information to understand disease pathogenesis, distinguishes EOOP from other bone fragility disorders, and guides in patient management, but is not widely available in clinical settings. Despite the great advances in this field, EOOP remains an insufficiently explored entity and further research is needed to optimize diagnostic and therapeutic approaches. © 2022 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: OSTEOPOROSIS; GENETIC RESEARCH; WNT/β-CATENIN/LRPS; BONE MODELING AND REMODELING; BONE HISTOMORPHOMETRY

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Introduction

Osteoporosis, characterized by deterioration of bone microstructure, low bone mineral density (BMD), and fractures, is a common disease in the elderly population and especially in postmenopausal women.[1,2] Because fractures often require hospital care and associate with increased mortality, osteoporosis has considerable human, social, and economic implications worldwide.[3,4] On rare occasions, osteoporosis may present already in childhood or early adulthood as a consequence of another condition (eg, chronic diseases of organs such as liver, kidney, lungs and bowel, chronic inflammatory diseases, endocrine diseases including hypogonadism, and neuromuscular disorders), hematological and oncological diseases, long-term medication (eg, glucocorticoid therapy, cancer treatment), or as a result of nutritional deficiencies (eg, anorexia nervosa, celiac disease, or deficient calcium or vitamin D intake).[1,5,6] In addition to these secondary forms of osteoporosis, skeletal fragility at an early age can result from a pathogenic variant in a gene playing a pivotal role in bone metabolism.[7,8] The diagnosis of early-onset osteoporosis (EOOP) encompasses all the above-mentioned forms of osteoporosis presenting in premenopausal women and in men <50 years of age.

Bone undergoes continuous cycles of formation and resorption through bone modeling and bone remodeling.[9] Bone modeling primarily occurs during childhood and adolescence and is responsible for skeletal growth and shaping of the bones in response to mechanical loading and other (eg, endocrine) factors.[10] Upon achievement of peak bone mass by early adulthood, bone mass remains stable in midlife and begins to decline thereafter; this decline is particularly rapid in women during a few years after menopause.[11] Bone remodeling, on the other hand, refers to the continuous renewal of bone tissue by the coupled activity of the three bone cell lineages: the osteoblasts, the osteoclasts, and the osteocytes.[12] The bone extracellular matrix (ECM), which is primarily composed of type I collagen and noncollagenous proteins, plays a pivotal role in bone remodeling by conferring mechanical support and providing growth factors to the bone cells. Impairment of any of these processes can lead to inadequate bone mass accrual and maintenance, predisposing to osteoporosis.[10]

Despite extensive research, EOOP remains an inadequately characterized entity. In this review we aim to provide clarity to diagnostic approaches and elucidate the clinical, genetic and bone tissue features of EOOP. We use selected monogenic forms of EOOP to highlight the complex nature of genetics behind EOOP and to underscore the differences in bone tissue characteristics in some genetic forms of EOOP. Comprehensive information on these aspects is needed for patient identification and optimal management.

Definition and Subtypes of EOOP and Path to Diagnosis

The term EOOP refers to osteoporosis occurring in children and young adults. In comparison to postmenopausal osteoporosis, the definition of osteoporosis and intervention threshold in younger individuals are less clear, and the relationship between BMD and fracture risk have not been well established. In growing children, the diagnosis of osteoporosis requires the presence of a significant fracture history and low BMD, as defined by international guidelines (Fig. 1*A*).[13] The first criterion is defined as a history of at least two fractures before the age of 10 years or three or more fractures before adulthood.[7] The latter criterion is defined as a BMD *Z*-score ≤ −2.0 of the spine or total body. However, the presence of one or more spinal compression fractures in absence of major back trauma constitutes osteoporosis, even if BMD is normal.[7,13,14] Only fractures resulting from low-to-moderate energy events are considered. Furthermore, minor fractures (eg, fingers, toes, nose) are not considered when evaluating fracture history.[7] However, despite these diagnostic criteria, the clinical situation should be considered individually in each child and adolescent with suspected osteoporosis, as anticipated disease course and medications may require osteoporosis management even before the diagnostic criteria are fulfilled.[6]

In adults, the diagnosis of EOOP can be given to premenopausal women and males <50 years of with a low BMD, defined as a BMD *Z*-score ≤ −2.0 or *T*-score ≤ −2.5 at the lumbar spine or femoral neck,[1] when associating with either fragility fractures or an underlying chronic illness (Fig. 1*A*). It is noteworthy that routine DXA studies are not performed in this age group but a dual-energy X-ray absorptiometry (DXA) scan is usually indicated after two or more fragility fractures (due to low-to-moderate energy trauma), after a fracture at an unusual site such as the spine or hip, or in the presence of a chronic illness or medication predisposing to osteoporosis.[1,15,16]

Overall, EOOP is a relatively rare condition, especially when not associated with an underlying chronic disease or other secondary factors (Fig. 1*A*).[1,7] Exclusion of such secondary causes may require extensive assessments because numerous clinical situations may lead to bone fragility (Fig. 1*B*). More extensive lists and descriptions of secondary factors linked to osteoporosis have been described elsewhere.[15-18] Moreover, EOOP can sometimes manifest with extraskeletal features, including ocular and neurological impairments.

To understand the cause of primary EOOP, genetic testing is recommended, especially if the disease manifests at a very young age or if there is a family history for osteoporosis. In unclear situations, after exclusion of secondary factors and especially if no pathogenic variants in the genes presently linked to bone fragility are identified or a new gene-disease association is suspected, a bone biopsy may be considered for research purposes. Histology and histomorphometry of transiliac bone biopsy samples could give indication on the type of osteoporosis (eg, low-turnover versus high-turnover osteoporosis), unveil a mineralization defect (osteomalacia), or reveal rare causes of osteoporosis like mastocytosis or multiple myeloma. Bone biopsy analysis can also guide in diagnostics and in choosing the therapy, in cases where secondary osteoporosis has been excluded, but this method is not widely used in clinical settings. These aspects are discussed in detail in this review. Despite comprehensive search for secondary causes or genetic defects, the cause of EOOP may remain unknown and such forms are called idiopathic (Fig. 1*A*).

Osteogenesis imperfecta (OI) is the best-known genetic bone fragility disorder and, especially in its mild forms, is sometimes challenging to differentiate from other forms of EOOP. OI is a heterogenous condition mainly characterized by low BMD and frequent fractures, skeletal deformities, and disproportionate short stature.[19] However, a spectrum of other features, including blue sclerae, dentinogenesis imperfecta, hearing loss, scoliosis, joint laxity, and cardiopulmonary impairments are often found in patients with OI. The first clinical classification of OI by Sillence and colleagues[20] in 1979 divided OI into four types based on the severity of clinical features, ranging from mild OI with blue sclerae and a low number of fractures to perinatally

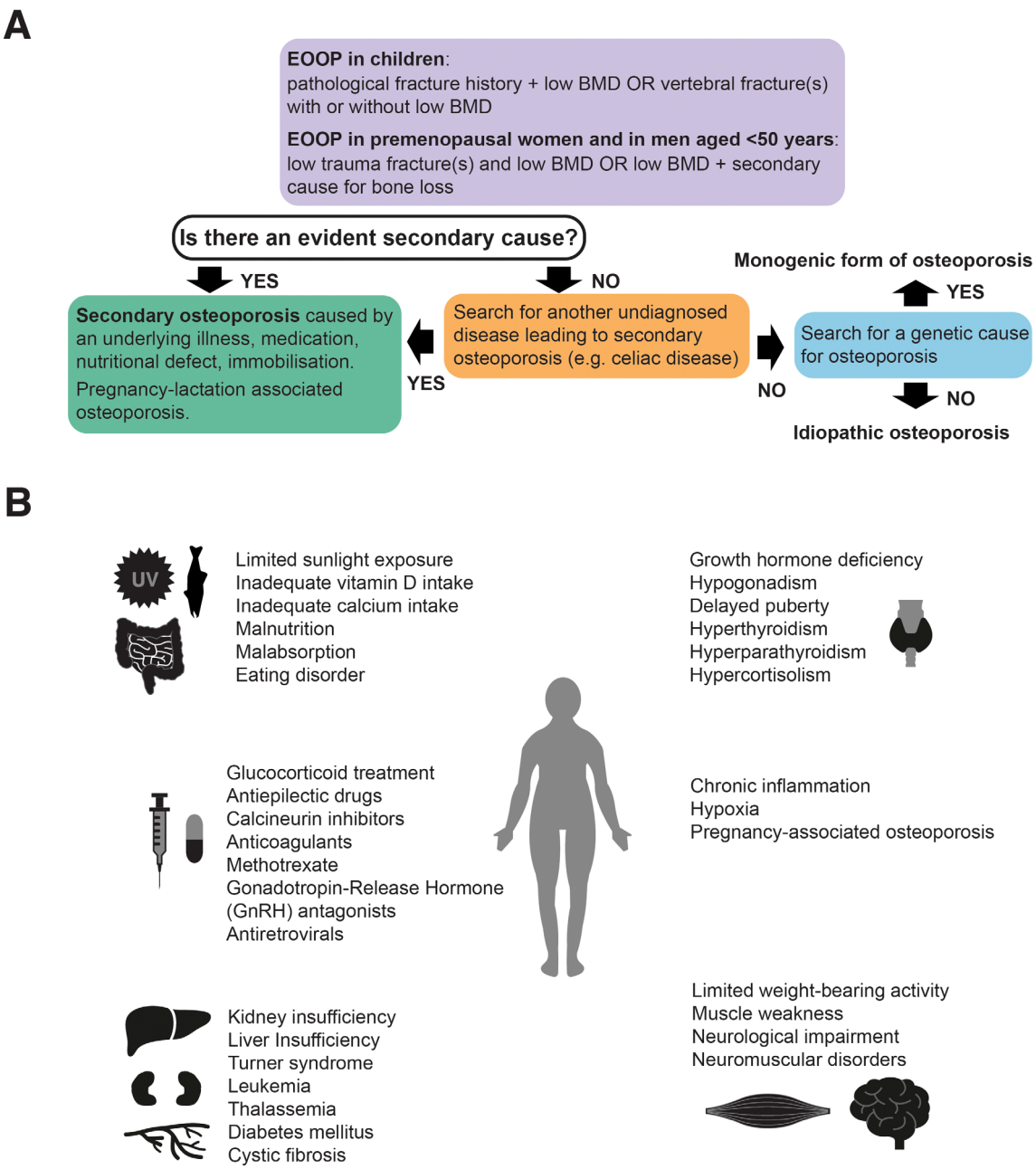

Fig. 1. (*A*) Definition of EOOP and diagnostic path. (*B*) Summary of the main causes of secondary osteoporosis.

lethal forms. Approximately 85% of OI cases are caused by pathogenic variants in the two genes encoding type I collagen, *COL1A1* and *COL1A2*.[21] Collagen type I defects can be either quantitative (low amount but normal quality of type I collagen due to, eg, stop gain mutations) or qualitative (abnormal type I collagen structure, eg, due to a missense mutation). Normally, qualitative defects lead to more severe phenotypes than changes in protein amount because they impair the structure of the collagen fibrils.[22] Recently, the phenotypic and genetic spectrum of OI has considerably expanded and pathogenic variants in at least 16 other genes have been identified.[23,24] Most of these genes play a pivotal role in synthesis, posttranslational modification, and processing of type I collagen.[22,23,25,26] This heterogeneity in disease spectrum has complicated the disease classification and has led to challenges in defining each clinical entity in the Online Mendelian Inheritance in Man (OMIM) catalogue. In this catalogue, each disease has a unique phenotype MIM number, but occasionally more than one phenotype MIM number is linked to the same gene. For instance, biallelic variants in *WNT1* are linked to "OI" (MIM 615220) whereas monoallelic variants in the same gene are associated with "susceptibility to osteoporosis" (MIM 615221).

In addition to OI, several other rare syndromes featuring bone fragility as a part of a broader phenotype have been identified.

For example, osteoporosis-pseudoglioma syndrome (OPPG, MIM 259770) and spondylo-ocular syndrome (MIM 605822) are both characterized by severe childhood-onset skeletal fragility and ocular manifestations.[23,24] Further, various mineralization defects, such as hypophosphatasia (HPP) due to pathogenic variants in the gene encoding tissue-nonspecific alkaline phosphatase (*ALPL*),[27] or various inherited forms of hypophosphatemia, due to defective renal phosphate handling, lead to skeletal fragility.

Although the clinical classification of OI still largely follows the original Sillence classification,[28] the International Nosology of Genetic Skeletal Disorders[23] groups the various forms of OI and other primary skeletal fragility disorders into one category. Presently, all forms of osteoporosis presenting at an early age are often included under the umbrella term of EOOP. However, because several well-defined conditions may present with early-onset skeletal fragility, one should aim at establishing a specific diagnosis whenever possible, instead of using the diagnosis EOOP for all such cases.

In the present review our main focus is not the classical OI and the rare genetic forms directly linked to defective type I collagen, or the various mineralization disorders. Instead, we have chosen to highlight other less well-known monogenic forms and idiopathic forms of EOOP to underscore the complex genetic and molecular architecture of EOOP, which is also reflected to the tissue-level manifestations and bone material properties.

Clinical Vignettes

The following two patient descriptions highlight the diagnostic path and challenges in young patients with an unusual fracture history and suspected osteoporosis.

Patient 1

A 36-year-old woman was diagnosed with osteoporosis after developing back pain following her third pregnancy. Spinal radiographs indicated vertebral compression fractures in the thoracic spine. She had a height loss of 4 cm. Otherwise the fracture history was negative. She had earlier been diagnosed with atrophic gastritis and received vitamin B12 injections every 2–3 months. She had also undergone thyroidectomy for Hashimoto's thyroiditis and thyroid nodules and received adequate thyroxine replacement therapy.

After the initial diagnosis of osteoporosis based on DXA scanning, she was treated with alendronate until the age of 42 years. Her family history was positive for osteoporosis and fractures, prompting genetic studies for OI. Sequencing of *COL1A1* and *COL1A2* detected no pathogenic variants, and the cause of osteoporosis remained unknown. At 44 years she was again investigated for osteoporosis to determine the underlying cause. Clinical evaluation showed normal facial features with white sclerae, normal dentition, and absent ligament laxity or skeletal deformities. In addition to height loss, now at 7 cm, she had kyphosis and mild scoliosis. Radiographs showed significant osteopenia in long bones and multiple thoracic vertebral compression fractures in the thoracic spine (Fig. 2*A*). DXA showed a BMD *Z*-score of −2.9 at the lumbar spine, −1.0 at femoral neck, and −1.7 at the total body. Serum concentrations for calcium, phosphate, parathyroid hormone (PTH), 25-OH-vitamin D and thyroid parameters were normal, as were bone turnover markers urine N-terminal telopeptide (U-NTX), serum N-terminal propeptide of type I collagen (S-P1NP), and serum C-terminal telopeptide of type I collagen (S-ICTP). Due to her severe and unexplained spinal osteoporosis and fractures at young age, a transiliac bone biopsy was obtained and confirmed the diagnosis of osteoporosis, showing low bone volume, no osteomalacia, and very low bone turnover.

The family history was again reviewed in detail and considered indicative of an autosomal dominant inheritance pattern with several male and female relatives, including the patient's mother and aunt, developing EOOP in childhood or early adulthood. Therefore, despite the history of pregnancy and lactation preceding osteoporosis and some chronic illnesse possibly contributing to bone fragility, a monogenic form of EOOP was considered. Linkage analysis followed by targeted sequencing lead to identification of a heterozygous missense mutation p.(Cys218Gly) in the *WNT1* gene. *WNT1* variants were not yet linked to EOOP at the time of analysis. The same mutation was confirmed by Sanger sequencing in all other affected family members.[29,30] Because of the low bone turnover and the earlier, apparently unsuccessful, treatment with bisphosphonates, she was treated with teriparatide for 2 years with a modest increase in bone turnover markers but no significant improvement in BMD or bone quality (Fig. 2*A*).[31] Although the biopsy was taken 2 years after bisphosphonate treatment, the previous medication may have contributed to low turnover rate and also to the suboptimal response to teriparatide.

Patient 2

A 27-year-old man experienced a hip fracture after a moderate-energy trauma (Fig. 2*B*). He had sustained one previous fracture after a fall during childhood. DXA measurements were indicative of osteoporosis with a lumbar spine BMD *T*-score of −3.0; femoral neck BMD showed osteopenia. Spinal radiographs and magnetic resonance imaging (MRI) revealed mild anterior wedging of two vertebral bodies (Fig. 2*B*). These findings confirmed the presence of EOOP and prompted further investigations. Secondary causes, such as underlying endocrine or hematological diseases, were excluded (Table 1). The only remarkable finding was a relatively low serum alkaline phosphatase (ALP) of 36 U/L (Table 1). Diagnosis of HPP was thus considered. In addition to low ALP, HPP leads to increased levels of ALP substrates such as serum pyridoxal phosphate (vitamin B6) and urinary phosphoethanolamine.[32,33] These substrates were not elevated, making the diagnosis of HPP unlikely but not completely excluded. To clarify the diagnosis, a bone biopsy was taken at the posterior iliac crest. It confirmed osteoporosis with normal mineralization but very low bone turnover. This was consistent with the finding of a low serum ALP despite normal values of the bone turnover markers beta-isomerized type I collagen C-terminal telopeptide (β-CTx) and P1NP.

These prompted evaluations for genetic causes of his osteoporosis. The family history for osteoporosis was negative. No extraskeletal or OI-type features were present. A targeted gene panel for 41 known genes linked to OI and other monogenic bone fragility disorders found no likely pathogenic variants nor variants of undetermined significance in these candidate genes (Fig. 2*C*), including *ALPL*. The etiology thus remained unknown and he was diagnosed with idiopathic osteoporosis, in line with the patient's young age, presence of low BMD and fragility fractures, absent secondary causes and no identified genetic cause,[1,15,16] and supported by the findings in the bone biopsy.[34]

Treatment guidelines for this age group remain unestablished. Modification of lifestyle factors with exercise, healthy nutrition and adequate intake of calcium and vitamin D are advocated and when these measures are insufficient and fracture risk regarded

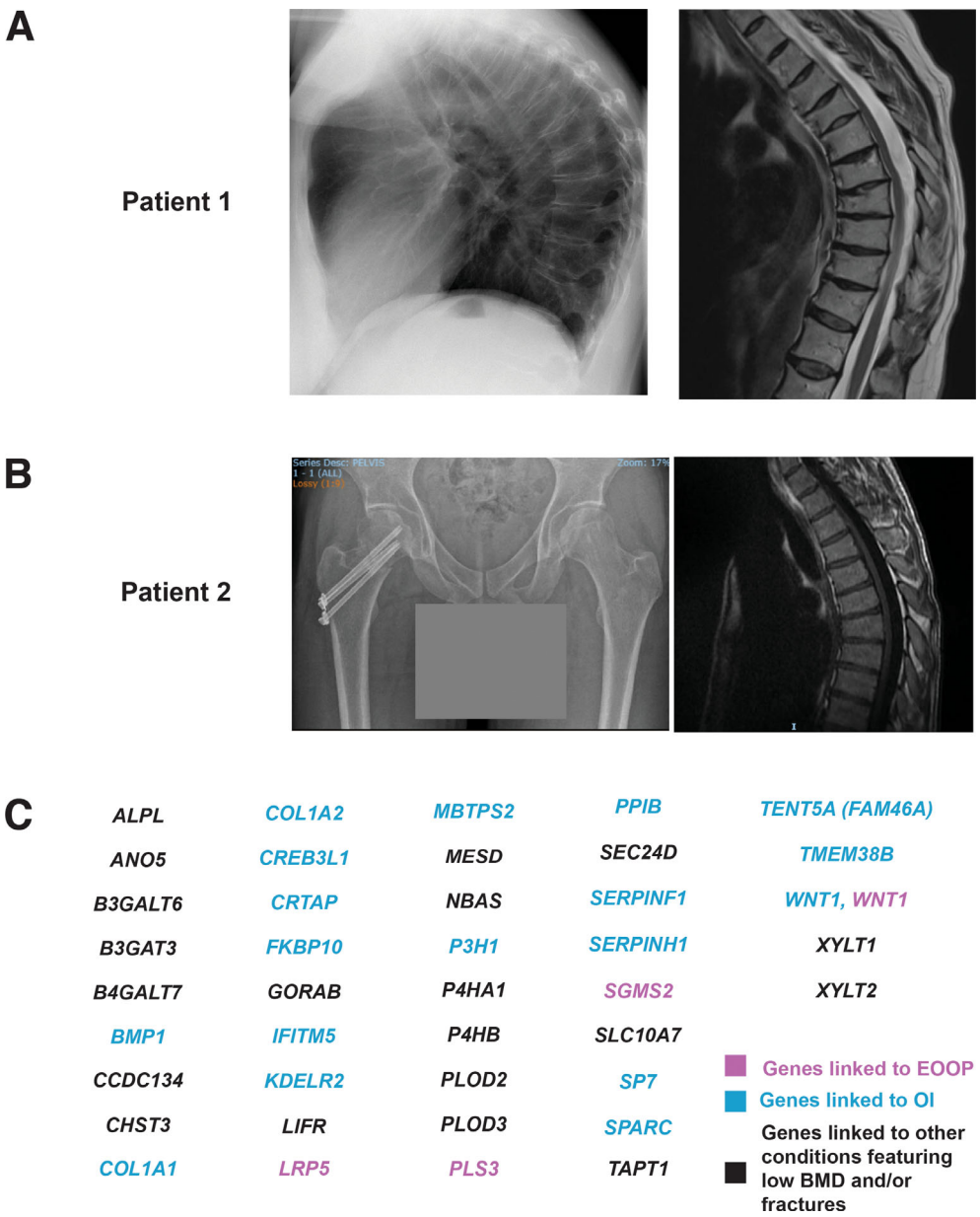

Fig. 2. (*A*) Radiograph of patient 1 at 44 years of age (left) showing generalized osteoporosis with multiple compressions, narrow disk spaces and anterior lipping of the narrow disk spaces; increased thoracic kyphosis. Magnetic resonance imaging (MRI) of the thoracic spine 9 years later (right), at the start of teriparatide treatment, shows progression of the vertebral compression fractures in the thoracic spine. (*B*) Radiograph of patient 2 (left) showing that the femoral neck fracture was surgically corrected. The pelvis and femurs appear osteopenic. Spinal MRI (right) showing multiple vertebral compression fractures. (*C*) Gene panel used for patient 2.

as high, antiresorptive and anabolic drugs can be used. Due to his very low BMD and low bone turnover, teriparatide treatment was initiated and is still ongoing. Given the inherent low bone turnover, need for antiresorptive medication after the planned 2-year treatment with teriparatide remains to be seen.

Approaches to Genetic Diagnostics in EOOP

Until not so long ago, a tentative diagnosis of bone disorders was based on a detailed clinical characterization and extensive literature review followed by testing for a specific candidate gene.[35] With the exploding number of known disease genes and the possibility for parallel testing of several genes by next generation sequencing (NGS), the field of genetic diagnostics has adopted a strategy that is often called "sequencing first."[36] In EOOP, a solid clinical and biochemical phenotypic characterization remains crucial for establishing a correct diagnosis. However, genetic testing is recommended, especially if the disease manifests at a very young age or if there is a family history of osteoporosis.

There are several approaches to identify the disease-specific candidate gene variants. Screening of individual sequences by Sanger sequencing has become the exception and reserved for

Table 1. Laboratory Evaluations for Excluding Secondary Osteoporosis in Patient 2

Parameter	Result	Reference range
Calcium	2.45 mmol/L	2.20–2.65 mmol/L
Phosphate	1.11 mmol/L	0.8–1.40 mmol/L
Alkaline phosphatase	36 U/L	<115 U/L
Tryptase	4.8 µg/L	<11.4 µg/L
Vitamin B6	101 nmol/L	35–110 nmol/L
TSH	1.83 mU/L	0.26–4.27 mU/L
PTH	0.8 and 1.2 pmol/L	0.68–4.40 pmol/L
Testosterone	19.7 nmol/L	10–30 nmol/L
25-OH Vitamin D	120 nmol/L	50–120 nmol/L
b-CTx	0.53 µg/L	<1.0 µg/L
P1NP	72 µg/L	19.4–95.4 µg/L
BALP	11.3 µg/L	<20.1 µg/L
Calcium (in urine)	6.0 mmol/L per 24 hour	2.5–7.5 mmol/L per 24 hour

b-CTx = beta-isomerized type I collagen C-telopeptide; BALP = bone-specific alkaline phosphatase; P1NP = procollagen type I N-propeptide; PTH = parathyroid hormone; TSH = thyroid stimulating hormone.

hotspot variants causing well recognizable disorders; eg, achondroplasia due to the frequent p.(Gly380Arg) variant in *FGFR3*[37,38] or Caffey disease due to the recurrent p.(Arg836Cys) change in *COL1A1*.[39] If no such single candidate gene or variant is known, NGS gene panels can be used (Fig. 3). They can comprise dozens to several hundreds of genes associated with a certain disease group or phenotype.[40] Although there are also amplification-based gene panels, in most cases the sequences to be analyzed are isolated by enrichment using complementary, sequence-specific probes (Fig. 3). However, EOOP-specific genes alone are not sufficient to fill such a gene panel and are usually combined with genes linked to overlapping disorders, such as OI or disorders of bone mineralization (Fig. 2C),[23,26] until the total enriched sequence reaches a reasonable size, usually 50–500 kilobases (kb). Because of the limited size, each gene in such a panel is usually well covered and the results are very reliable. Phenotype-specific custom-designed panels are expensive and therefore standardized. Larger gene panels, which are not indication-specific, have also been developed and may contain, eg, all known genes for monogenic disorders (OMIM genes). Currently, 4532 OMIM genes covering roughly 9 megabases (Mb) are known and require a sequencing output of 1 gigabases (Gb) for a good coverage. Although such panels may be technically unproblematic, determining the relevance of the panel findings for the individual patient may prove challenging. Moreover, all commercial laboratories use different gene panels and these must be repeatedly updated as novel disease genes are discovered. This problem is avoided with whole-exome sequencing (WES).[41] The exome is a large gene panel (around 45 Mb) enriching all exons of all 20,000 genes known so far (Fig. 3). Differences exist between exome versions; eg, some focus mainly on the coding sequences of verified protein-coding genes and others comprise additional genes and more noncoding sequences. Exomes have gaps; for example, they only partially cover most of the introns. An advantage of using WES is the possibility of identifying novel gene-disease associations.

Although most disease-coding variants are exonic or located nearby in upstream or downstream regions, deep intronic variants leading to abnormal splicing can also be disease-causing. A potential approach to identify noncoding variants influencing either gene expression or splicing is RNA sequencing.[42] However, bone disease genes are often not expressed by readily available leukocytes, thus fibroblasts derived from a skin biopsy are needed as RNA source, limiting the use of this more invasive method in clinical settings due to ethical and patient-related issues. Therefore, the all-in-one solution for coding and noncoding variants as well as structural variants (mostly copy number variants) is whole-genome sequencing (WGS) (3 Gb) (Fig. 3).[43] Although routine diagnostics has largely been taken over by gene panels and WES, WGS is likely to become the standard within the coming years also in the clinical settings,[44] and especially in research searching novel disease genes.

Sequencing of 36 Mb of exome target sequence reveals around 90,000 single-nucleotide variants (SNVs), or approximately one SNV in 400 base pairs (bp). This magnitude of

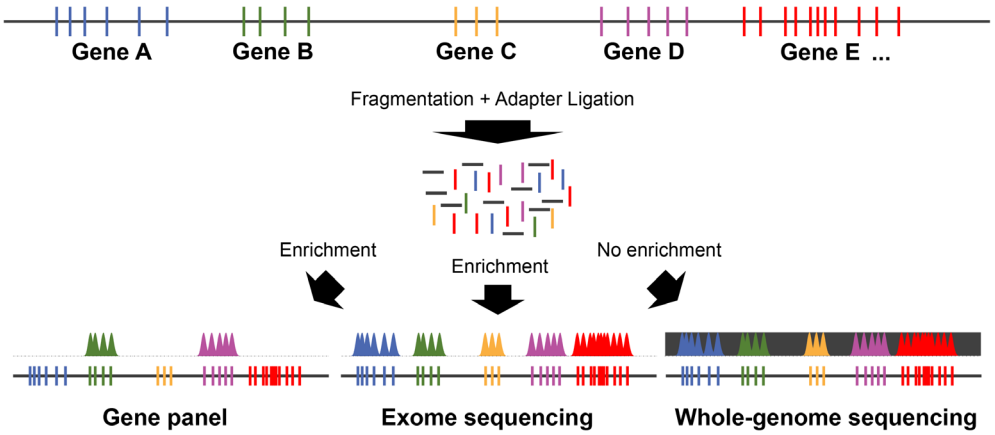

Fig. 3. Different NGS-based diagnostic approaches. A schematic genomic region is depicted containing five genes with different numbers of exons (colored bars). Interspersed noncoding sequences are represented in dark gray. After fragmentation of the entire genomic DNA and ligation of sequencing adapters the enrichment strategy determines which part of the genome is sequenced. Gene panels can be of different sizes, the exome contains all protein-coding genes plus variable noncoding and putative genes, and the genome most of the coding and noncoding sequences.

variants can only be interpreted with the help of bioinformatics.[45] Software tools allow filtering of variants according to their frequency, predicted or known pathogenicity, inheritance pattern, and phenotype relevance. Only with such information their significance can be determined. However, phenotype information as well as family history are crucial for the interpretation process and an integral factor in the filtering strategy based on the human phenotype ontology (HPO).[46,47] Once the genetic cause is identified, it is possible to provide genetic counseling and guide in disease surveillance and management.

Unfortunately, the significance of a single variant often remains uncertain and many variants are determined as "of unknown significance" (VUS). In the clinical settings it is not possible to functionally test each variant and it remains a task for advanced research to establish which variants in specific genes could lead to disease manifestations.[48]

Sequencing family trios (index patient and parents) is more powerful than sequencing the sole index case because several variants can be discarded if they do not fit the inheritance pattern. In addition, WES and WGS also offer the possibility of combining the samples into cohort-wide burden tests using collapsing analysis frameworks.[49] Finally, WES and WGS data can be analyzed retrospectively. Due to the large size of the data, the use of NGS technologies requires high computational capacity and large data storage systems. Storing NGS data not only has high costs but it might also lead to ethical issues due to the presence of sensitive data.

Although the costs of genetic tests have decreased during the recent years, NGS, especially WGS, is still an expensive tool and not all clinics have the possibility to use this method on a routine basis. For this reason, the decision on the approach chosen for investigating a family with EOOP not only depends on the clinical findings but also on the financial resources of the laboratory/clinic.

Rare Genetic Forms of EOOP Highlight Complexity of Disease Pathogenesis

Although the International Skeletal Dysplasia Society (ISDS) Nomenclature includes a large number of monogenic conditions featuring bone fragility,[23] most of the disorders in this category represent various types of OI with defects in type I collagen metabolism or other well-defined entities with specific extraskeletal manifestations, such as ocular impairments like in spondyloocular syndrome or cutaneous symptoms like in geroderma osteodysplasticum (MIM 231070). Only a handful of other monogenic forms of EOOP have been characterized. We have chosen to focus on these genetic diseases in which low BMD and fractures are the main features. The discussed genetic forms of EOOP include *LRP5* and *WNT1*, involved in the WNT signaling pathway, plastin-3 (*PLS3*), and sphingomyelin synthase 2 (*SGMS2*). These highlight the diversity in molecular pathology in EOOP and describe proteins with very diverse functions that are often not restricted to bone metabolism. These genetic conditions further underscore the importance of genetic testing in arriving at an exact diagnosis, for differentiating between clinically similar skeletal fragilities, and for considering possible extraskeletal symptoms related to the underlying molecular defect. Some of these monogenic forms were identified only recently and their pathomolecular mechanisms remain elusive.

Defects in the WNT signaling pathway

WNT signaling is one of the key pathways governing skeletal health—from early skeletal development to bone mass accrual throughout childhood and to bone mass maintenance and bone metabolism in adulthood.[50-52] The deleterious skeletal consequences of aberrant WNT signaling are exemplified in several human skeletal disorders[29,53-59] and the integral role of this pathway for bone homeostasis has been validated by extensive functional studies in both cell and animal models.[60-66]

The landmark discovery was the identification of biallelic loss-of-function mutations in the gene for low-density lipoprotein (LDL) receptor-related protein 5 (*LRP5*) in patients with OPPG.[53,67] This disease presents in early childhood with low BMD and multiple peripheral and vertebral fractures, often involving the whole spine. The skeletal features are accompanied by congenital or infancy-onset blindness ("pseudoglioma") secondary to defective vascularization. LRP5 was identified as the key transmembrane co-receptor for WNT ligands and the gatekeeper for the intracellular cascade regulating osteoblast function and bone formation[52,68,69] (Fig. 4*A-C*). Variants in the *LRP5* gene were subsequently linked to several bone mass abnormalities of both low and high bone mass, including phenotypes of EOOP in subjects with monoallelic loss of function (LOF) variants in *LRP5* (MIM 166710).[55]

Compared to patients harboring biallelic variants, carriers of *LRP5* variants feature a milder phenotype, characterized by low BMD and fractures but without severe eye impairments or with only mild vitreoretinopathy.[53,70] Studies have identified monoallelic *LRP5* variants in individuals with childhood or early-adulthood onset symptomatic osteoporosis.[55] In addition, both rare and common *LRP5* variants have been associated with childhood bone mass, peak bone mass, and propensity to fractures[55,71-73] and, in genomewide association studies (GWASs), with BMD and fractures in the general population.[72] Some larger cohort studies on EOOP have identified various monoallelic *LRP5* variants in affected adults, but the true contribution of these variants to disease pathogenesis, without functional evidence, has often remained uncertain.[74] Pathogenic *LRP5* variants have also been described in patients developing EOOP during pregnancy and lactation.[70] Another study involving >350 individuals with EOOP, diagnosed based on low BMD, identified *LRP5* or *LRP6* variants in 8.3% of patients.[74] Individuals carrying rare *LRP5* or *LRP6* variants had low bone turnover markers, in line with decreased WNT signaling[74] but overall there was significant heterogeneity in skeletal manifestations and response to osteoporosis treatment in those harboring a variant.

Only years after the discovery of *LRP5*'s role in osteoporosis, WNT1 was identified as a key ligand to the canonical WNT pathway in bone (Fig. 4*A,B*). We and others reported on biallelic or monoallelic *WNT1* variants in subjects with low BMD and prevalent spinal and peripheral fractures.[29,57-59] Monoallelic *WNT1* variants cause a milder dominantly inherited phenotype with EOOP, peripheral and vertebral compression fractures, and loss of adult height (MIM 615221) whereas biallelic *WNT1* variants result in a much more complicated phenotype reminiscent of OI with severe skeletal fragility, long-bone deformities, kyphoscoliosis, and short stature (MIM 615220). After the first publications, several cases with biallelic *WNT1* mutations have been reported,[75-78] confirming the severe OI type III-like clinical phenotype in affected individuals, who often show ptosis, a specific hallmark of this disease.[75]

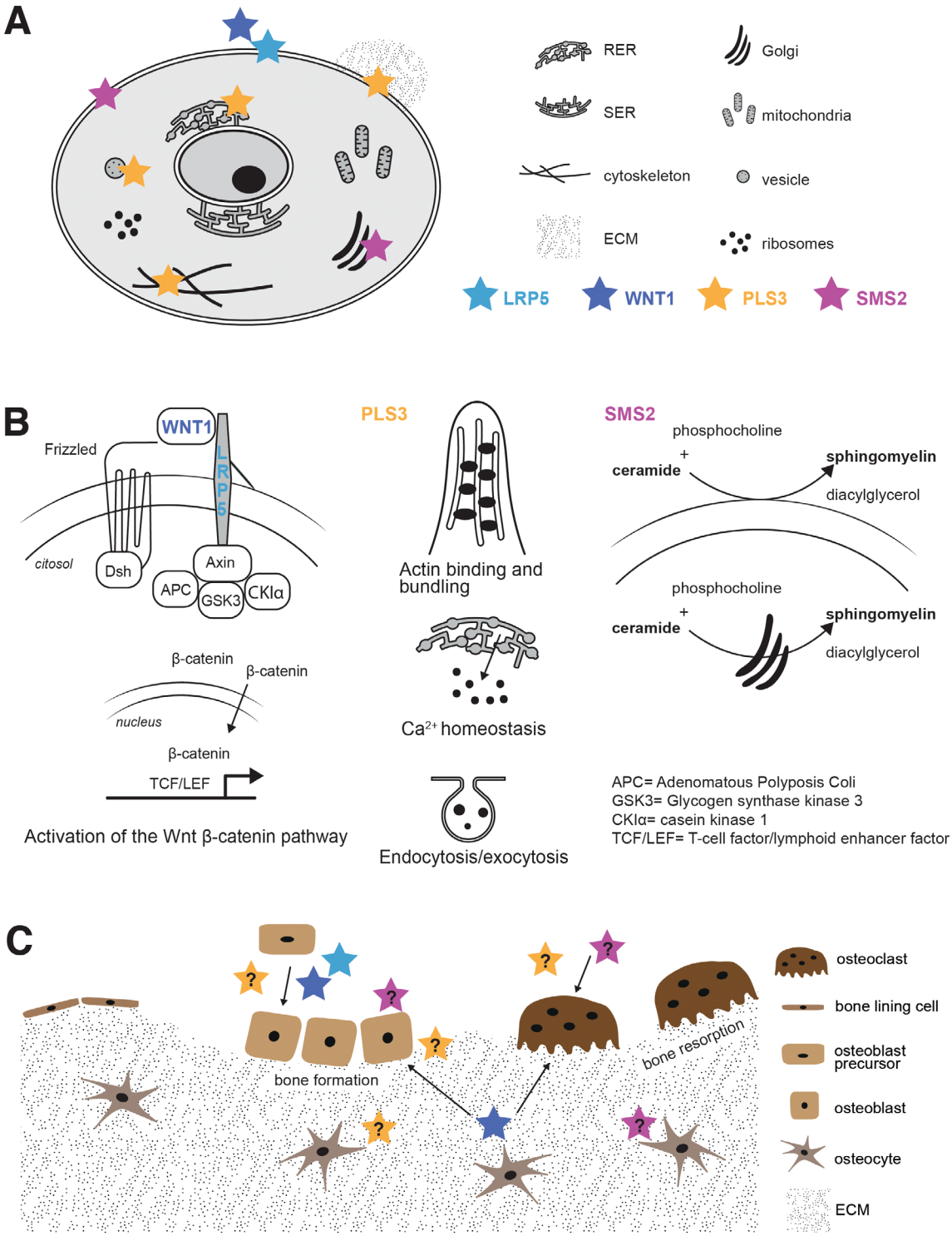

Fig. 4. EOOP due to LRP5, WNT1, PLS3, and SMS2 defects. (*A*) Subcellular localization of the proteins encoded by *LRP5*, *WNT1*, *PLS3*, and *SGMS2*. (*B*) Molecular pathways involved in EOOP. (Left) When the WNT1 ligand binds to the receptor seven-span transmembrane Frizzled and the co-receptor LRP5, Dishevelled (Dsh) is recruited and the β-Catenin destruction complex (composed by axin, APC, GSK3, and CK1α) is inactivated thus leading to the accumulation of β-Catenin in the cytosol. Subsequently, β-Catenin translocates to the nucleus and activates the transcription of target genes, including those responsible for osteoblast differentiation and function. Monoallelic loss-of-function variants in *WNT1* and *LRP5* lead to osteoporosis due to decrease activation of the canonical WNT signaling pathway. (Center) Plastin-3, encoded by *PLS3*, is involved in several molecular mechanisms, including actin binding and bundling, calcium homeostasis, and cell endocytosis and exocytosis. (Right) The sphingomyelin synthase 2 (SMS2), encoded by *SGMS2*, is involved in sphingomyelin synthesis primarily in the plasma membrane but also in the Golgi apparatus. (*C*) LRP5 (light blue star), WNT1 (dark blue star), PLS3 (yellow star), and SMS2 (pink star) are involved in bone modeling and remodeling. Although both WNT and LRP5 are known to regulate osteoblast differentiation and function, the role of the other proteins is not fully understood yet. PLS3 might be involved in osteocyte mechanosensing, matrix mineralization, but also osteoblast differentiation and osteoclastogenesis. SMS2 could also partake in matrix mineralization, osteoblast activity, and osteoclastogenesis.

Despite the fact that the phenotype in autosomal recessive WNT1 skeletal fragility is welldescribed, the full spectrum of clinical features associated with monoallelic *WNT1* variants is less well characterized, with only less than 20 families reported to date (Table S1). We have described two large unrelated Finnish families with a monoallelic missense variant p.Cys218Gly and consequently EOOP with prevalent peripheral fractures in childhood and vertebral fractures later in adulthood, with otherwise normal growth and development.[29] In our cohort, comprising 25 mutation-positive individuals aged 11 to 76 years, the age-of-onset, fracture susceptibility, and disease progression were greatly variable. This is supported by other reports, describing patients with heterozygous *WNT1* variants with normal to osteopenic BMD, no fractures, and normal quality of life, but also individuals with considerable skeletal fragility, hypotonia, joint hypermobility, delayed motor skills, and developmental abnormalities requiring extensive rehabilitation.[59,79] Isolated findings include gastrointestinal symptoms, strabismus, and neurological symptoms.[79]

Although the significance of WNT1 ligand for bone homeostasis is well-established, the exact underlying molecular mechanisms remain unclear.[80] WNT1 partakes in the crosstalk between osteoblasts and hematopoietic stem cells to regulate bone cell development, differentiation, and proliferation in the developmental stages, and later, in adult bone, is secreted from osteocytes and activates adjacent osteoblasts and osteocytes in a paracrine manner (Fig. 4*C*).[64] Net yield from the activated pathway is anabolic, leading to increased bone formation and decreased bone resorption.[50,64,81] Loss of Lrp5 in mice did not reduce the bone-anabolic effect of Wnt1, thus suggesting that this co-receptor is not needed for Wnt1 function.[80] Aberrant WNT1 signaling in patients seems to primarily reduce cortical bone thickness due to decreased periosteal bone formation, whereas trabecular bone formation and rate of bone resorption are less affected.[81] These are congruent to findings in patients' bone biopsies with low number of bone cells and low bone turnover rate.[29,82] In-depth immunohistochemistry of bone biopsies revealed increased fibroblast growth factor 23 (FGF23) expression, increased marrow adiposity, apoptotic osteocytes in cortical bone, and abnormalities in osteocyte morphology in individuals with prior bisphosphonate treatment.[83]

Optimal means for diagnosis and follow-up of *WNT1* patients are unknown. Despite the severely disturbed bone metabolism, serum concentrations of conventional metabolic bone markers are unchanged.[82,84] An extensive biomarker survey found significantly elevated serum concentrations of both intact and C-terminal FGF23 while, interestingly, the two osteocytic inhibitors of WNT signaling, dickkopf-related protein 1 (DKK1) and sclerostin, were normal.[84] Further, screening of serum samples for a panel of 192 common microRNAs (miRNAs) distinguished a unique miRNA signature in *WNT1* mutation-positive subjects with nine differentially expressed miRNAs.[85]

Plastin-3 related osteoporosis

In 2013, the same year that *WNT1* was discovered, a Dutch group reported on an X-linked form of inherited primary osteoporosis presenting predominantly in males (MIM 300910).[86,87] This form of EOOP was caused by hemizygous variants in the X-chromosomal gene encoding plastin-3, *PLS3*. The disease is defined in males by low BMD, multiple peripheral fractures, and especially spinal compression fractures in the thoracic spine since young childhood, leading to progressive kyphosis, severe spinal pathology, and height loss already by early adulthood.[25,88-90] Mild extraskeletal features may also be present, such as facial dysmorphism, joint hypermobility, waddling gait, grayish sclerae, hearing loss, and opalescent teeth.[86,87,91-98] Due to its X chromosomal inheritance pattern, males are more severely and earlier affected than females.[93]

To date, less than 30 pathogenic LOF *PLS3* variants have been identified, mostly frameshift variants[99] (including some partial and whole-gene deletions[92,94,100] and an intragenic duplication[101]) and nonsense variants,[99] although some missense changes[99] have also been found in individuals with EOOP. No genotype–phenotype correlation is observed and the variants affect both the regulatory domain and the four calponin homology domains of *PLS3*.[99] Both frameshift variants and missense mutations can lead to severe osteoporosis.[99] Moreover, common *PLS3* variants have been associated with osteoporosis in postmenopausal women.[86,102]

In subjects with PLS3-related EOOP, basic parameters of calcium homeostasis are normal and bone turnover markers are either normal or slightly reduced,[84,87,94,103] as compared with age-matched mutation-negative controls. However, *PLS3* mutation-positive subjects had elevated DKK1 concentrations, suggesting that PLS3 impairment might also alter WNT signaling in bone. Similar to patients with WNT1 osteoporosis, seven miRNAs that are predicted to play a role in bone were found to be significantly upregulated or downregulated in patients with pathogenic *PLS3* variants.[84,104]

Even if PLS3 has an undeniably important role in bone metabolism, the underlying molecular mechanisms, however, remain elusive. Plastins 1–3 (PLS1–PLS3) are a family of proteins that bind to and crosslink actin structures into larger fibers[105] (Fig. 4*A,B*). PLS3, the most abundant plastin, has a wide expression range and is highly expressed in adipocytes and endothelial cells but nearly absent in the bone marrow (https://www.proteinatlas.org).[106] In general, PLS3 is more expressed in mesenchymal-lineage cells than in myeloid-lineage cells.[106] In contrast, PLS2 is selectively enhanced in bone marrow and lymphoid tissues, especially in monocytes, but has a very low expression in mesenchymal-lineage cells.[106] Finally, PLS1 is primarily found in intestinal tissues and nearly absent in the bone marrow.[106] PLS3 is an F-actin binding and bundling protein that is involved in cytoskeletal remodeling (Fig. 4). However, this protein might also have roles in calcium homeostasis,[107] vesicular trafficking, endocytosis, and exocytosis[99] (Fig. 4). Interestingly, other medical conditions not affecting bone are also associated to *PLS3* defects.[99] For example, high PLS3 expression is associated with different types of hematological and solid cancers, including acute myeloid leukemia and colon cancer,[108-110] but it is also found to be a protective modifier for spinal muscular atrophy.[111]

Although PLS2, which is highly expressed in hematopoietic cell lineages, has a function in osteoclast actin ring formation, movement, and bone resorptive activity,[100,112-114] the information on the role of PLS3 in bone is still limited (Fig. 4*C*). A recent study by Yorgan and colleagues[115] showed that a *Pls3* knockout mouse model features reduced cortical thickness without low BMD and a decreased mineralization capacity by osteoblasts. In contrast, Neugebauer and colleagues[114] reported reduced cortical thickness with osteoporosis due to increased bone resorption in *Pls3* knockout mice. However, the effects of *PLS3* overexpression or knockout on osteoclasts are very modest.[114] The fact that anti-resorptive treatment in patients with PLS3-related osteoporosis increases BMD, suggests that

osteoclasts are active in PLS3-related osteoporosis.[84,87,100] PLS3 has also been suggested to play a role in osteocytes' mechanosensing and mechanotransduction (Fig. 4*C*). Osteocyte shape is dependent on actin filaments and osteocyte processes are rich in actin.[116] Moreover, mechanical forces generated by the ECM are transferred inside the cells through integrins, propagated to filaments of F-actin and finally transmitted to the nucleus.[117] In chicken, PLS3 is located in the dendrites of the osteocytes, supporting a role in mechanotransduction.[118] Therefore, PLS3 could be especially important for osteocyte function (Fig. 4*C*).

PLS3 has also been implicated in bone matrix mineralization. Transiliac bone biopsy samples in children and adults with *PLS3* mutations show low bone turnover, often associated with increased osteoid[89,93] and high cortical osteocyte apoptosis,[83,94] uneven mineralization pattern in childhood, and a more uniform increase in mineralization in adults.[31,94] During the mineralization process, matrix vesicles are formed by budding from the mineralizing cell microvilli. These contain, as a structural core, a dense bundle of cross-linked actin microfilaments. In a mineralizing osteocyte-like cell line PLS3 was present both in the budding matrix vesicles and in the apical microvilli from which the vesicles were formed.[119] It can be hypothesized that defective PLS3 function disturbs this matrix vesicle-mediated mineralization process, leading to defective mineralization that can be seen in patient-derived bone biopsies. Nevertheless, the number of analyzed patients as well as the number of controls is limited. Moreover, PLS3-osteoporosis arises during childhood, but the so far investigated bone biopsies are taken at an adult age. For this reason, some bone surfaces have already disappeared, thus this may imply a risk for potential bias when performing histomorphometric analyses.

Defects in sphingomyelin synthase 2

In 2019, we reported a novel disease gene underlying a rare skeletal disorder, "osteoporosis with calvarial doughnut lesions" (OP-CDL; MIM 126550). OP-CDL was clinically described already in 1990s,[120] followed by several reports of similarly affected patients, but the genetic cause remained unknown until years later. Our study confirmed that monoallelic mutations in *SGMS2*, encoding sphingomyelin synthase 2 (SMS2), are responsible for OP-CDL.

Sphingomyelin is a major lipid of the plasma membrane and is enriched in microdomains, lipid rafts, of the plasma membrane that are critical for signal transduction. SMS2 is an enzyme that resides primarily in the plasma membrane,[121,122] where it synthesizes sphingomyelin from ceramide and phosphatidylcholine (Fig. 4*A*,*B*).

The disease manifests as childhood-onset osteoporosis with low BMD and frequent fractures. However, a much more severe skeletal dysplasia was also associated with the same gene. To date, three different *SGMS2* variants have been identified and the disease severity varies depending on the underlying variant.[123,124] Although the recurrent nonsense variant p.Arg50* associates with a milder phenotype featuring EOOP, the missense variants p.Ile62Ser and p.Met64Arg result in a more severe phenotype of spondylometaphyseal dysplasia with multiple spinal and peripheral fractures, scoliosis, and severe short stature.

Regardless of the *SGMS2* variant, patients also exhibit a peculiar cranial feature of multiple sclerotic, doughnut-shaped skull lesions. Although the radiographically observed lesions seem to be confined to the skull, radiographs portray similar uneven mineralization in long bones with alternating areas of increased density and osteopenic appearance.[123] Furthermore, bone biopsies from affected patients show gross disturbance and inconsistency in bone matrix mineralization.[123,125] Our detailed analyses of transiliac bone biopsies from two *SGMS2* variant-positive adult males with EOOP depicted an overall decrease in bone volume with disorganized arrangement of collagenous fibrils (woven bone appearance) and disruption in organization and continuity of the osteocyte–canalicular network and osteocyte orientation.[125] Moreover, we showed that eroded surface to bone surface ratio (ES/BS) was highly elevated in both patients, while the osteoclast surface to bone surface ratio (Oc. S/BS) showed variable outcomes.[125] Although another study also revealed reduced cortical volumetric BMD and thickness in another two patients with OP-CDL,[124] the number of osteoclasts and the extent of eroded surface in the iliac crest were slightly reduced in these patients.[124] Patient-derived osteoclasts, induced form CD14+ cells, did neither differentiate nor resorb differently.[123] Similar to WNT1 and PLS3-related osteoporosis, markers of bone metabolism are normal but circulating levels of alkaline phosphatase tend to be elevated.[123]

Several features of this form of EOOP remain unclear but some hypotheses can be drawn from existing data. The cranial lesions, which seem to be confined to the calvarial bones, are an interesting feature of the disease. These lesions seem to be absent in childhood and appear during adulthood. They also seem to grow in size and become more lytic-appearing with increasing age. Interestingly, the sclerotic/lytic calvarial lesions share some resemblance with calvarial lesions observed in tumor metastasis.[126,127] Such bone lesions are triggered by a vicious cycle, which to a large extent is driven by growth factors and inflammatory cytokines.[128] In fact, SMS2 has been linked to inflammatory responses and total suppression or inhibition of SMS2 reduces the inflammatory response in several specific conditions.[129-131] Furthermore, osteoclasts originate from hematopoietic stem cells like immune cells such as macrophages and monocytes,[9] and considering the tight interplay between immune cells and osteoclasts,[132] there might be a link between the patients' sclerotic lesions and osteoclast function. Inhibition or knockdown of SMS2 attenuates the permeability of endothelial cells *in vitro* and *in vivo*.[129,133] One can hypothesize that this could potentially cause reduced oxygen tension contributing to osteolysis. While a study by Yan and colleagues[134] identified *SGMS2* as a regulatory gene of late embryonic craniofacial development in mice, the restriction of lytic lesions to the skull bones in OP-CDL still remains unclear.

Although osteoblasts have been reported to be negatively affected by SMS2 impairment, the information is still sparse. Matsumoto and colleagues[135] reported that sphingomyelin synthase 1, but not SMS2, is involved in bone formation by osteoblasts in mice. Moreover, Yoshikawa and colleagues[136] reported that *Sgms2* knockdown in primary murine osteoblasts reduces the expression of the nuclear factor κB (NF-κB) ligand and increases the expression of osteoprotegerin, thus decreasing osteoclastogenesis. However, these studies do not reflect the situation in humans because SGMS2 deficiency leads to osteoporosis, patchy mineralization, cortical trabecularization, and calvarial osteolytic/doughnut lesions.[123-125]

Differential diagnosis between various monogenic forms of EOOP

Despite differences in pathophysiology, it is evident that the described forms of monogenic osteoporosis have substantial

overlap and may have greatly varying clinical presentations regarding age of onset, disease severity, and treatment response. Further variability is introduced by different variants in the same gene, as seen in *SGMS2*-related pathology. Taking into account further changes introduced by aging, lifestyle factors, and comorbidities, the clinical presentation can be easily obscured and unclear even to an experienced clinician.

With increasing clinical data from newly diagnosed patients, subtle trends in skeletal characteristics are observed, especially regarding sites of fracture, bone deformities, spinal involvement, and cranial abnormalities. *PLS3* variants seem to have a major impact on the spine, resulting in vertebral compressions and long bone fractures already at a young age. Vertebral compression fractures lead to thoracic kyphosis and loss of adult height. Similar vertebral complications are seen in WNT1 osteoporosis, although usually only later in adulthood and not in childhood.[137,138]

Although the severe form of OP-CDL manifests as severe long bone and spinal changes, patients with the nonsense variant p.Arg50* tend to have milder vertebral changes but suffer from frequent long-bone fractures. These typically present in early childhood and subside by adulthood. Peripheral fractures are also common in patients with WNT1 osteoporosis; they often occur in childhood but become even more prevalent in adulthood.

Second to differences in skeletal characteristics are the presence and array of possible extraskeletal features. The affected genes are often ubiquitously expressed, regulate a wide range of developmental and cellular processes, and may therefore predispose to other organ manifestations. WNT1, for one, has a key role in the central nervous system and in patients harboring *WNT1* mutations neurological manifestations such as severe cerebellar hypoplasia, epilepsy, cerebellar, pontine, and mesencephalic tectum hypoplasia, severe global developmental delay, and gross brain atrophy have been reported.[58,139] Although these are usually diagnosed in patients with biallelic *WNT1* variants, it is possible that even patients with a monoallelic *WNT1* variant could manifest milder neurological features.

Neurologic features are also a central feature in OP-CDL.[123] Alongside skeletal manifestations, *SGMS2* mutation-positive subjects may present with recurrent and transient facial, trochlear, and oculomotor nerve palsies that are isolated and spontaneously remitting. Other recorded features include hearing loss, sensory neuropathy with progressive ataxia, and gross developmental delay. Despite the skull lesions, the symptoms are not secondary to cranial sclerosis and extensive clinical evaluations or brain imaging studies have found no structural changes, suggesting that the changes in nerve cell function arise directly from abnormal neuronal sphingomyelin metabolism.

Bone Biopsy as a Tool to Explore Bone Tissue Characteristics in EOOP

Beyond bone mass and geometry, alterations affecting bone material properties at different levels of hierarchy can reduce the capacity of bone to dissipate the energy of an impact without fracturing.[140] Transiliac bone biopsy samples offer a unique and microscopic look into changes in bone material properties and can be obtained for diagnostic purposes to evaluate histology and histomorphometry in unclear pathological conditions.[141,142] In "classical" OI cases with genetically confirmed type I collagen defects, bone biopsy is usually not indicated as there are treatment guidelines. In other forms of primary EOOP the analysis of bone biopsy may provide important insight into pathogenesis and guide also in disease management. Double tetracycline-labeled bone biopsies may be indicated when the cause of the osteoporosis or fractures is unclear, when the presentation is unusual or fractures continue despite therapy. Biopsies help to differentiate between osteomalacia and osteoporosis and between high and low bone turnover states.[142] Transiliac crest bone biopsies are also useful to evaluate treatment effects (Table 2). This tool may not be readily available in clinical practice but has been widely used in research settings.

The residual bone sample block can be used for further analyses such as quantitative backscattered electron imaging (qBEI) to gain information on mineralized bone volume, the degree of mineralization of the ECM and two-dimensional (2D) osteocyte lacunae sections (OLS) characteristics (Table 2).[143-145] This technique utilizes the linear correlation between the intensity of the backscattered signals and the calcium content of the ECM.[143,146-150] Other methods to analyze bone tissue properties at the microscale and nanoscale, such as small-angle X-ray scattering to determine size and orientation of the mineral particles,[151-155] and vibrational spectroscopy (Fourier-transform infrared or Raman)[156,157] to investigate the structure of the organic matrix or a mechanical investigation using, eg, nanoindentation,[158] are reported elsewhere. For many of these methods, reference values for healthy individuals (children and adults) are available.[141,143,145,152,159-164]

To elucidate the variability of bone tissue features in different forms of EOOP and to underscore the considerable amount of information obtained from bone biopsy with different analytic methods, we discuss here bone material properties based on the evaluation of transiliac bone biopsy samples in two representative pediatric patients. One patient has EOOP due to a monoallelic *WNT1* variant and the other child has typical OI due to a *COL1A1* variant leading to a quantitative defect in type I collagen. Although the net result of these variants in both cases is increased bone fragility, the tissue pathology differs considerably. As shown in Fig. 5, qBEI microscopy of bone biopsy samples indicated a reduction of 40% in trabecular mass in the patient with OI[160] compared to a healthy girl,[161] whereas this parameter was within lower normal limits in the child with EOOP due to a *WNT1* variant[31] (Fig. 5*A*). In OI, the low trabecular bone volume is associated with abnormal bone remodeling; bone histomorphometric analyses have shown that bone turnover is increased in OI due to elevated numbers of osteoblasts and osteoclasts, whereas ECM production at the single cell level is markedly reduced. This metabolic imbalance leads to poor increase in trabecular thickness and increased resorption of the bone ECM.[159,165] Consistently, the nearly normal trabecular bone volume in the child with a *WNT1* variant is in line with normal indices of bone formation and resorption found in this and other children with *WNT1* variants.[31,166]

The qBEI microscopy image can be translated into a gray-level histogram corresponding to a bone mineralization density distribution (BMDD) curve (Fig. 5*B*). This distribution arises from the fact that bone ECM is not homogenously mineralized, but rather consists of a mosaic of bone packets with variable mineral content depending on local tissue age. Younger bone packets have a lower mineral content than older ones and changes in bone turnover, as well as disturbances in the mineralization process, will have a profound impact on the shape of the BMDD.[167,168] In healthy children and adolescents, the BMDD for trabecular bone shows little variation with age, sex, ethnicity, and skeletal site.[161,169-171] Any deviation from this physiological window is

Table 2. Overview of bone tissue characteristics in transiliac bone biopsy samples and effect of treatment for OI type I and EOOP due to *LRP5*, *WNT1*, *PLS3*, and *SGMS2* defects. The results have been compared to reference data from healthy children and adolescents.[141,145,161]

	OI type I	***LRP5***	***WNT1***	***PLS3***				***SGMS2***	
	n=19[145,159,160]	n=1[197]	n=1[31,83]	n=2[87]	n=1[94]	n=1[195]	n=2[31,83]	n=2[123]	n=1[124]
Age (years, y) Gender (male, m; female, f)	2.2-14.1y 13m/6f	6y/m	14y/m	4y/m 8y/m	9.7y/m	12y/m	9y/m 13y/m	12 y/f 15 y/m	5y/f*
Histology and histomorphometry:[141]									
Cortical width	↓	↑	↔	↓	↓	↓	↓	↔, ↔	↔
Trabecular bone volume/tissue volume (%)	↓	↓	↔	↓	↓	↓	↓	↔, ↓	n.a
Osteoid thickness (μm)	↔	↔ Tb ↔ Ct	↔	↓	↑	↓	↔	↔, ↑	↔
Osteoid surface/bone surface (%)	↑	↓ Tb ↔ Ct	↔	↓	↑	↓	↔	↔, ↑	↔
Osteoblast surface/bone surface (%)	↑	n.a	↔	↔	↔	↓	↔	↓, ↔	↑
Mineralizing surface/bone surface (%)	↑	↓ Tb ↔ Ct	↔	↔	↓	↓	↔	↔; n.a	↑
Mineral apposition rate (μm/d)	↓	↓ Tb ↔ Ct	n.a	↔	↑	↓	n.a	↔; n.a	↓
Bone formation rate/bone surface ($\mu m^3/\mu m^2$/year)	↔	↓ Tb ↔ Ct	n.a	↔	↓	↓	↔	↔; n.a	↔
Bone formation rate/osteoblast surface (%/year)	↓	n.a	n.a	n.a	n.a	n.a	n.a	n.a	↔
Mineralization lag time (days)	↔	n.a	n.a	n.a	↑	↓	n.a	↑, n.a	↑
Osteoclast surface/bone surface (%)	↔	n.a	↔	↔	↔	↓↓	n.a	↔, ↔	↔
Eroded surface (%)	↔	↔	↔	n.a	↓	↓	n.a	↔, ↑	↔
Lamellar (LB)/Woven bone (WB)	LB	n.a	LB	n.a	n.a	LB	LB	WB	n.a
Bone mineralization density distribution by quantitative backscattered electron microscopy:[143,161]									
Mean calcium concentration (CaMean, weight % Ca)	↑	↔ Tb ↑ Ct	↓	↔	↓	↑	↔, ↓ Tb ↔, ↔Ct	↓	n.a
Most frequent calcium concentration (CaPeak, weight % Ca)	↑	↔ Tb ↑ Ct	↓	↔	↓	↑	↔, ↓ Tb ↔, ↓ Ct	↓, ↓ Tb; ↑, ↓ Ct;	n.a
Heterogeneity in mineralization (CaWidth, Δ weight % Ca)	↓	↑ Tb ↔ Ct	↑	↔	↑	↔	↑	↑, ↑ Tb ↑, ↑ Ct	n.a
Percentage of lowly mineralized bone areas (CaLow, % bone area)	↔	↑ Tb ↓ Ct	↑	↔	↑	↓	↔, ↑ Tb ↔, ↔ Ct	↑, ↑ Tb; ↑, ↑ Ct;	n.a
Percentage of highly mineralized bone areas (CaHigh, % bone areas)	↑	↑ Tb ↑ Ct	↓ Tb ↔ Ct	↔	↓	↑	↔	↔, ↔ Tb; ↑, ↔ Ct;	n.a
Osteocyte lacunae sections (OLS) characteristics by quantitative backscattered electron microscopy:[145]									
Porosity (%)	↑	n.a	↑	n.a	n.a	n.a	n.a	↑	n.a
Density (number/mm^2)	↑	n.a	↓	↑	n.a	n.a	n.a	↑	n.a
Area (μm^2)	↓ Tb ↔ Ct	n.a	↑	n.a	n.a	n.a	n.a	↑	n.a

Tb: trabecular bone, Ct: cortical bone; ↑: increased; ↓: decreased; ↔: within normal limits

*Histomorphometry outcomes were obtained from cortical bone (trabecular bone was not available) and reference values are from.[196]

potentially deleterious and contributes to bone fragility; hypermineralization stiffens the bone material and makes it more brittle, whereas hypomineralization softens it and makes it weaker.

BMDD in the patient with the *COL1A1* variant reveals a pronounced hypermineralization (BMDD curve shifted to higher mineralization) corresponding to high-turnover OI, whereas a BMDD shift to lower mineralization indicative of low-turnover EOOP is found in the patient harboring the *WNT1* variant (Fig. 5*B*). Normally, low to normal bone remodeling rates lead to normal or increased mineralization and high bone turnover rates to lower mineralization.[143,168] The fact that exactly the opposite is the case in OI and WNT1 osteoporosis, indicates that in both cases, not only the turnover rate but also the mineralization kinetics are disturbed.

Because mineral homeostasis, mechanosensing, and bone remodeling are largely controlled by osteocytes,[172-174] the quantitative assessment of osteocyte parameters such as size and density might provide important insights into material abnormalities. The fingerprint of dead osteocytes are highly mineralized lacunae. This phenomenon, known as "micropetrosis," is believed to hamper mechanosensitivity and the repair of bone microdamage, contributing to bone fragility.[175,176] Hypermineralized osteocyte lacunae are easily identifiable on qBEI as bright dots. So far, no increase in micropetrosis has been found in healthy children or in pediatric patients with EOOP.[169,177] Figure 5*C* shows that OLS-porosity is high in the child with OI whereas it appears within normal range in the child with the *WNT1* variant. Patients with a decreased OLS-density and an increased OLS-area may still show normal OLS-porosity, and conversely, osteocyte dysfunction might be associated with abnormal OLS-density and/or abnormal OLS-size.[31,83] The OLS analysis of bone sections is a 2D method, whereas a full three-dimensional (3D) assessment of osteocyte lacunar volume and shape can be achieved using X-ray tomography.[178]

Goldner trichrome staining (Fig. 5*D*) allows to distinguish mineralized ECM (stained in green) from osteoid (stained in red) and evaluate structural parameters as well as osteoblast and osteoclast activity.[141] In addition, Goldner-stained histological sections can be viewed under polarized light to assess collagen fibril orientation. Such analyses allow to distinguish primary disordered woven bone from remodeled lamellar bone. Woven bone is less mature, more cellular, and more prone to fracture.[179] Woven bone formation is characteristic for severe forms of OI and less frequent in the rather mild OI type I,[180] but present also in OP-CDL.[123] However, even lamellar bone can be of reduced quality if less or defective collagen is secreted, lamellae and collagen fibrils are thinner, and crosslink formation altered.[19,21,157,159,180]

As discussed in this article, bone biopsy is a valuable tool that provides a large amount of tissue-level information about the pathology leading to bone fragility. However, this tool may not be readily available in clinical practice due to its invasive nature, large patient volume, and required expertise for biopsy analysis. Bone biopsies are widely used in research settings and these findings can hopefully in the future be translated to general management guidelines in the specified monogenic forms of EOOP, as already is the case with OI. Another downside is the incompleteness of normative data. Although such data are available for several parameters for healthy children and for classical OI, it remains to be established for other monogenic forms across age groups.

Treatment Strategies in Monogenic Forms of EOOP

Due to the paucity of clinical studies in young patients with osteoporosis, there are presently no specific evidence-based guidelines for treating EOOP. We provide here a concise summary of treatment strategies, focusing especially on the rare monogenic forms. For more extensive information, including management of nongenetic forms, the readers are advised to use other recent reviews specifically focusing on osteoporosis management in young patients and premenopausal women.[17,18,181,182]

As a first line of treatment, patients with EOOP should be given adequate calcium and vitamin D supplementation, if dietary evaluation or biochemistry shows insufficiency.[183,184] Furthermore, a healthy lifestyle, including good diet, regular exercise, no alcohol consumption, and no smoking, has been shown to improve BMD in both young healthy subjects and patients with EOOP.[185-187] An increase in BMD is also seen when secondary causes of EOOP are treated (eg, gluten-free diet for celiac disease).[16] When secondary EOOP is excluded but fragility fractures continue occurring, the use of antiresorptive and anabolic drugs should be considered.[1] Their effectiveness in children and young adults with EOOP has not been thoroughly studied due to the rarity of the condition and the fact that only small studies investigating effects on BMD but not on fractures have been carried out. This means that in each situation the potential benefits and side effects of medication will have to be weighed against the severity of bone fragility, also keeping in mind potential harms in women of childbearing age.

Prior studies have primarily focused on evaluating use of antiresorptive and anabolic drugs in collagen type I–related OI. In general, the use of bisphosphonates, such as pamidronate, alendronate, and zoledronate, to reduce osteoclast activity, increases BMD in patients with OI whereas its effect on fractures is less certain.[188,189] Clinical studies concerning denosumab, a monoclonal antibody inhibiting osteoclast activation, remain scarce and further investigation is needed to evaluate its full potential.[190] Teriparatide treatment leads to increased BMD and prevents spinal compression fractures in OI patients,[191,192] particularly in patients with mild OI compared to patients with severe forms.[191] Preclinical studies on two other anabolic drugs, sclerostin antibodies and transforming growth factor β (TGF-β) inhibitors to promote bone formation, resulted in increased BMD in different mice models of OI.[193,194] Clinical studies for testing anabolic molecules in OI patients are ongoing (clinical trials BPS-804 and NCT03064074).

However, as discussed earlier, results from studies involving patients with OI cannot be directly extrapolated to treatment approaches in EOOP as the bone tissue characteristics and bone turnover differ greatly. Using bisphosphonates in low bone turnover states may not be beneficial and hence personalized approaches need to be adopted when treating patients with monogenic forms of EOOP. However, although osteoporosis linked to *LRP5* and *WNT1* variants associates with decreased WNT signaling and decreased osteoblast activity and function, for EOOP due to *PLS3* and *SGMS2* variants the underlying pathomolecular mechanisms are still incompletely understood. Teriparatide treatment showed accelerated bone turnover in an adult male patient with the *LRP5* polymorphism p.Arg1036Gln[74] and in three adult male patients with the *WNT1* missense variant p.Cys218Gly.[30] Osteoporosis medication in nine patients with WNT1-related EOOP did not ameliorate their spinal

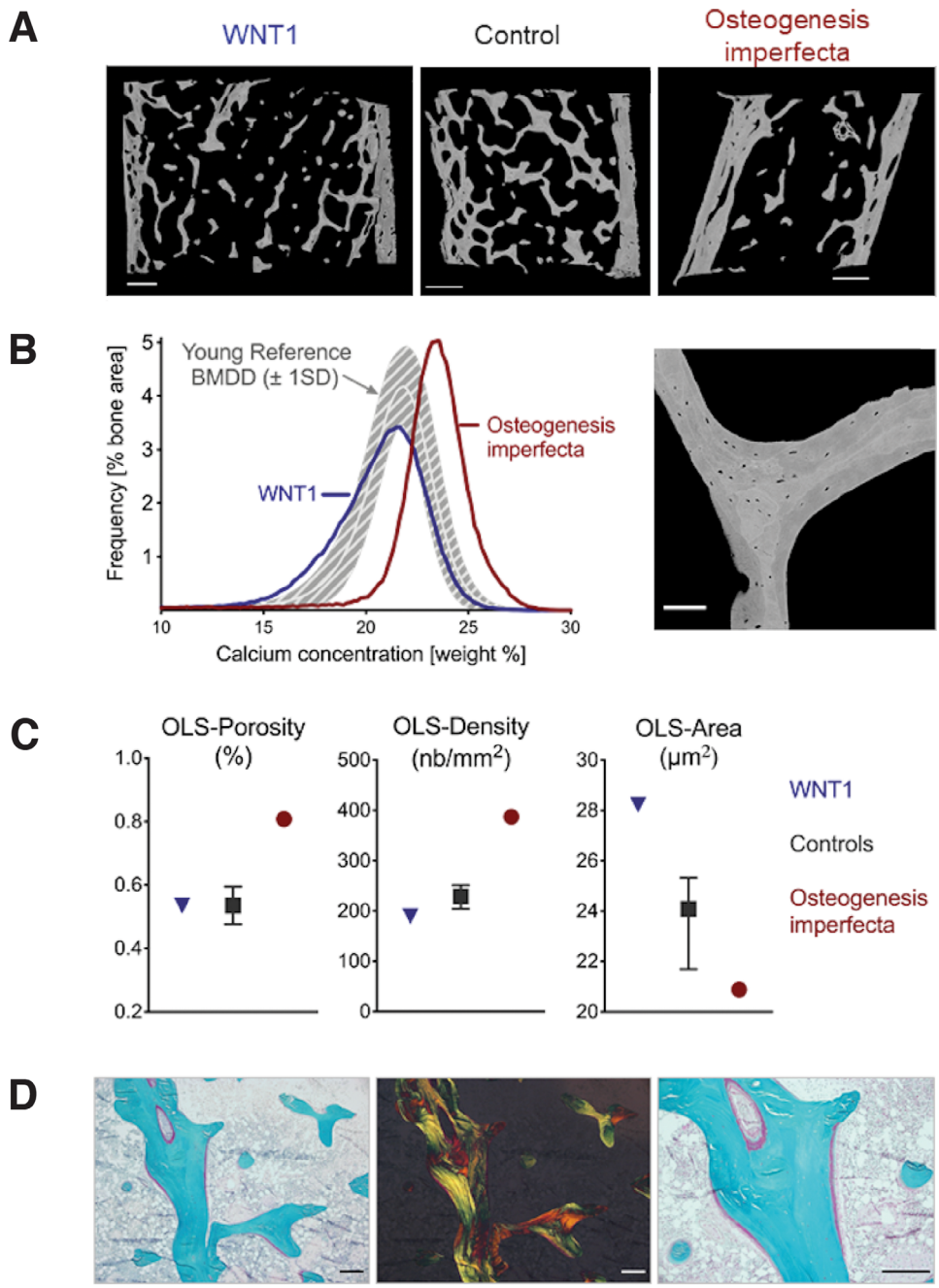

Fig. 5. (*A*) qBEI of selected bone biopsy samples: from a patient with a monoallelic *WNT1* variant (left; reproduced with permission from Elsevier[31]), from a healthy control (middle; reproduced with permission from Springer[160]) and from a patient with OI due to a *COL1A1* variant (right; reproduced with permission from Springer[160]). The inner and outer cortex and the trabecular compartment in between can be visualized. The trabecular bone volume is strikingly reduced in OI bone. The different gray scales relate to different calcium content of the ECM (Scale bar = 1 mm). (*B*) (Left) Corresponding BMDD curves: these represent frequency distributions of gray (calcium) levels and can be described by five parameters: the average and the most frequent calcium concentration (CaMean and CaPeak), the proportion of lowly and highly mineralized ECM (CaLow and CaHigh, respectively on the left and right side of the histogram) and the heterogeneity in mineralization, mirrored by the width of the histogram at half maximum (CaWidth).[143] Compared to the reference BMDD from healthy children (gray band), the BMDD from the child with OI is shifted to the right, towards higher matrix mineralization, while the BMDD curve in the patient with *WNT1* variant is shifted to the left, towards lower mineralization.[31,160,161] (Right) qBEI of a trabecular feature at high magnification (0.88 µm/pixel) to assess the osteocyte lacunae sections (OLS). Scale bar = 100 µm, published under a creative common attribution license.[145] (*C*) Results from the OLS-analysis. Compared to healthy references[145], bone of a patient with OI shows a large OLS-density and a reduced OLS-area, while the opposite is true for bone from WNT1 patients. The decrease in density and increase in area leads to an unremarkable OLS-porosity for WNT1 bone. (*D*) Thin section of bone from the patient with the *WNT1* variant stained with Goldner trichrome visualized with bright field microscopy (left and right) as well as under polarized light (middle). Under bright field large osteoid seams on the bone surface can be observed (red: osteoid, green: mineralized bone). Under polarized light a rather normal lamellar organization of bone ECM is found (Scale bars = 250 µm). BMDD = bone mineralization density distribution; OLS-porosity = percentage of total OLS area per bone area); OLS-density = number of OLS per bone area; qBEI = quantitative backscattered electron microscopy imaging.

pathology.[137] Considering the underlying defects, sclerostin antibodies might potentially be beneficial for these patients but clinical studies in this regard are not yet available.

Concerning PLS3-related EOOP, three adult patients (two males and one female) with the same splicing *PLS3* variant responded well to teriparatide treatment but the outcome on fracture risk has yet to be determined.[30] Moreover, bisphosphonate treatment in patients with pathogenic *PLS3* variants seems to increase BMD, improve vertebral shaping, and prevent new fractures.[86,93,100,101] Finally, the use of bisphosphonates in three children with SGMS2 osteoporosis prevented further long-bone fractures and improved back pain.[123]

Long-term effects of osteoporosis treatment in patients with *LRP5*, *WNT1*, *PLS3*, and *SGMS2* variants have yet to be evaluated. Moreover, clinical trials comparing EOOP patients and sex-matched controls need to be carried out to better investigate the effects of osteoporosis treatment and evaluate the safety of the use of these drugs in young patients. Finally, due to the diversity of mechanisms involved in EOOP, the variable and often inadequate response to the available osteoporosis treatments suggests that new personalized treatment approaches need to be identified and tested in larger patient cohorts. In order to identify novel treatment strategies, further studies on the molecular pathology of EOOP are needed.

Concluding Remarks

EOOP remains a rare but important clinical entity that poses challenges in diagnostic approaches and management. Several causative factors and mechanisms have been identified. Although the clinical features of EOOP overlap with other skeletal fragility syndromes, there are also critical differences relating to the underlying causes, clinical and bone-tissue characteristics, and choice of treatment. A thorough clinical investigation combined with family history and tailored biochemical tests are the first steps toward a diagnosis. When secondary causes of bone fragility are excluded, primary EOOP due to a constitutive genetic defect in a gene playing a pivotal role in bone metabolism should be considered. The broad use of NGS has sped up the identification of disease-causing variants and has also led to the identification of several novel genes linked to bone fragility. The pathomolecular mechanisms responsible for these recently identified forms of EOOP remain to be fully elucidated. Patient-derived bone biopsies provide a possibility to understand what the genetic defects cause at the tissue level, giving detailed information about bone structure, turnover, and mineralization. In the absence of large clinical studies on osteoporosis in young subjects, treatment guidelines for EOOP are lacking. Further research and novel personalized treatment strategies are required to optimize patient management.

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Alice Costantini: Conceptualization; data curation; funding acquisition; project administration; writing – original draft; writing – review and editing. **Riikka E. Mäkitie:** Conceptualization; data curation; writing – original draft; writing – review and editing. **Markus A. Hartmann:** Conceptualization; data curation; formal analysis; funding acquisition; writing – original draft; writing – review and editing. **Nadja Fratzl-Zelman:** Conceptualization; data curation; funding acquisition; methodology; writing – original draft; writing – review and editing. **M. Carola Zillikens:** Conceptualization; data curation; investigation; writing – original draft; writing – review and editing. **Uwe Kornak:** Conceptualization; data curation; methodology; writing – original draft; writing – review and editing. **Kent Søe:** Conceptualization; data curation; writing – original draft; writing – review and editing. **Outi Mäkitie:** Conceptualization; data curation; funding acquisition; project administration; supervision; writing – original draft; writing – review and editing.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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