Omics Profiling of S2P Mutant Fibroblasts as a Mean to Unravel the Pathomechanism and Molecular Signatures of X-Linked MBTPS2 Osteogenesis Imperfecta

Pei Jin Lim1,2†, Severin Marfurt1,2†, Uschi Lindert1,2, Lennart Opitz3, Timothée Ndarugendamwo1,2, Pakeerathan Srikanthan2,4, Martin Poms2,4, Martin Hersberger5, Claus-Dieter Langhans5, Dorothea Haas5, Marianne Rohrbach1,2* and Cecilia Giunta1,2‡

1 Connective Tissue Unit, Division of Metabolism and Children’s Research Centre, University Children’s Hospital, Zurich, Switzerland, 2 University of Zurich, Zurich, Switzerland, 3 Functional Genomics Center Zurich, University of Zurich/ETH Zurich, Zurich, Switzerland, 4 Division of Clinical Chemistry and Biochemistry, University Children’s Hospital Zurich, Zurich, Switzerland, 5 Department of Pediatrics, Centre for Pediatric and Adolescent Medicine, Division of Neuropediatrics and Metabolic Medicine, University Hospital, Heidelberg, Germany

Osteogenesis imperfecta (OI) is an inherited skeletal dysplasia characterized by low bone density, bone fragility and recurrent fractures. The characterization of its heterogeneous genetic basis has allowed the identification of novel players in bone development. In 2016, we described the first X-linked recessive form of OI caused by hemizygous MBTPS2 missense variants resulting in moderate to severe phenotypes. MBTPS2 encodes site-2 protease (S2P), which activates transcription factors involved in bone (OASIS) and cartilage development (BBF2H7), ER stress response (ATF6) and lipid metabolism (SREBP) via regulated intramembrane proteolysis. In times of ER stress or sterol deficiency, the aforementioned transcription factors are sequentially cleaved by site-1 protease (S1P) and S2P. Their N-terminal fragments shuttle to the nucleus to activate gene transcription. Intriguingly, missense mutations at other positions of MBTPS2 cause the dermatological spectrum condition Ichthyosis Follicularis, Atrichia and Photophobia (IFAP) and Keratosis Follicularis Spinulosa Decalvans (KFSD) without clinical overlap with OI despite the proximity of some of the pathogenic variants. To understand how single amino acid substitutions in S2P can lead to non-overlapping phenotypes, we aimed to compare the molecular features of MBTPS2-OI and MBTPS2-IFAP/KFSD, with the ultimate goal to unravel the pathomechanisms underlying MBTPS2-OI. RNA-sequencing-based transcriptome profiling of primary skin fibroblasts from healthy controls (n = 4), MBTPS2-OI (n = 3), and MBTPS2-IFAP/KFSD (n = 2) patients was performed to identify genes that are differentially expressed in MBTPS2-OI and MBTPS2-IFAP/KFSD individuals compared to controls. We observed that SREBP-dependent genes are more downregulated in OI than in IFAP/KFSD. This is coupled to alterations in the relative abundance of fatty acids in MBTPS2-OI fibroblasts.
INTRODUCTION

Osteogenesis imperfecta (OI), known more commonly as brittle bone disease, is a genetically heterogeneous disorder of bone matrix formation and remodeling and represents one of the most common forms of skeletal dysplasia (Krakow, 2015). OI is characterized by low bone density, recurrent fractures, bone deformities and short stature. Its additional features which include dentinogenesis imperfecta, progressive hearing loss and blue-gray hue of the sclerae demonstrate generalized connective tissue disorder and highlight the systemic nature of the disease presentation (Marini et al., 2017). The spectrum of severity of the clinical presentation of OI is broad, ranging from mild to severe forms with perinatal lethality. There is currently no cure for OI due to a yet incomplete understanding of its underlying pathomechanisms. The management of patients currently consists of a combination of supportive rehabilitation, orthopedic interventions to correct bone and joint deformities and pain management of acute fractures. The inhibition of bone resorption by intravenous or oral administration of bisphosphonates has become the mostly used pharmacological approach to reduce fracture rate in OI patients. However, bisphosphonate treatment is associated with a myriad of side effects, including osteonecrosis of the jaw and atypical femoral fractures, and the development of osteonecrosis of the femoral head (Rydevik et al., 2012). The use of bisphosphonates is therefore limited in OI patients, and other treatment strategies are urgently needed. Treatment in children and adults with OI who benefit from bisphosphonates has become the mostly used pharmacological approach to reduce fracture rate in OI patients. However, bisphosphonate treatment is associated with a myriad of side effects, including osteonecrosis of the jaw and atypical femoral fractures, and the development of osteonecrosis of the femoral head (Rydevik et al., 2012). The use of bisphosphonates is therefore limited in OI patients, and other treatment strategies are urgently needed.

The initial characterization of OI, known more commonly as brittle bone disease, is a genetically heterogeneous disorder of bone matrix formation and remodeling and represents one of the most common forms of skeletal dysplasia (Krakow, 2015). OI is characterized by low bone density, recurrent fractures, bone deformities and short stature. Its additional features which include dentinogenesis imperfecta, progressive hearing loss and blue-gray hue of the sclerae demonstrate generalized connective tissue disorder and highlight the systemic nature of the disease presentation (Marini et al., 2017). The spectrum of severity of the clinical presentation of OI is broad, ranging from mild to severe forms with perinatal lethality. There is currently no cure for OI due to a yet incomplete understanding of its underlying pathomechanisms. The management of patients currently consists of a combination of supportive rehabilitation, orthopedic interventions to correct bone and joint deformities and pain management of acute fractures. The inhibition of bone resorption by intravenous or oral administration of bisphosphonates has become the mostly used pharmacological approach to reduce fracture rate in OI patients. However, bisphosphonate treatment is associated with a myriad of side effects, including osteonecrosis of the jaw and atypical femoral fractures, and the development of osteonecrosis of the femoral head (Rydevik et al., 2012). The use of bisphosphonates is therefore limited in OI patients, and other treatment strategies are urgently needed.

In this follow-up study, we aim to further characterize the molecular features of MBTPS2-OI and compare it to that of IFAP/KFSD caused by MBTPS2 mutations (MBTPS2-Osteogenesis Imperfecta, connective tissue, transcriptomics, MBTPS2, site 2 protease

Keywords: X-linked osteogenesis imperfecta, connective tissue, transcriptomics, MBTPS2, site 2 protease

**MBTPS2** encodes the Golgi resident protein site-2 protease (S2P). S2P is a zinc metalloprotease involved in regulated intramembrane proteolysis (RIP), a process occurring on the Golgi membrane that activates several membrane-bound transcription factors. These include activating transcription factor 6 (ATF6), old astrocyte specifically induced substance (OASIS), box B-binding factor 2 human homolog on chromosome 7 (BBF2H7) and sterol regulatory element binding protein (SREBP) (Rawson, 2013). Upon RIP cleavage by S2P, the activated transcription factors that are released from the Golgi membrane translocate into the nucleus, where they exert their regulatory effects on the expression of genes involved in Endoplasmic Reticulum (ER) stress response (Ye et al., 2000), osteoblast differentiation (Murakami et al., 2009), chondrogenesis (Saito et al., 2009; Izumi et al., 2012), and lipid homeostasis (Horton et al., 2003), respectively. Notably, the activities of these transcription factors are induced by the RIP process when cells undergo ER stress (Ye et al., 2000; Murakami et al., 2006; Saito et al., 2009; Izumi et al., 2012; Zhang et al., 2012a; Rawson, 2013).

### Intriguingly, missense mutations at different positions of S2P cause distinct syndromes

Prior to its association with OI, pathogenic missense variants in MBTPS2 were described in the dermatological spectrum condition Ichthyosis Follicularis, Atrichia, and Photophobia (IFAP, OMIM 308205) and Keratosis Follicularis Spinulosa Decalvans (KFSD, OMIM 308800) (Oeffner et al., 2009; Aten et al., 2010; Bornholdt et al., 2013). MBTPS2 mutations causing IFAP/KFSD and OI may be located in proximity according to a predicted secondary structure of S2P (Figure 1) despite the lack of clinical overlap. This offers a unique biological context to explore the pathophysiology in the skeletal system by identifying mechanisms and molecules that are specifically perturbed in MBTPS2-OI but not in IFAP/KFSD and might be relevant to the development of bone and cartilage.

We previously showed that mutant S2P proteins are stably expressed in OI and IFAP/KFSD patient-derived fibroblasts, but cleavage of OASIS is impaired. Furthermore, co-transfection of mutant or wildtype MBTPS2 constructs and luciferase under the control of either ATF6 or SREBP in CHO-M19 cells lacking endogenous S2P demonstrated diminished ATF6 and SREBP activities upon ER stress induction in the cells expressing mutant S2P proteins. The data collectively suggest that S2P pathogenic variants in OI and IFAP/KFSD cause defective RIP and activation of the transcription factors (Lindert et al., 2016).

In this follow-up study, we aim to further characterize the molecular features of MBTPS2-OI and compare it to that of IFAP/KFSD caused by MBTPS2 mutations (MBTPS2-Osteogenesis Imperfecta, IFAP/KFSD) and healthy controls. In particular, we investigate whether molecular changes occur uniquely in response to pathogenic MBTPS2 variants causative of OI but not in response to variants causative of IFAP/KFSD, and whether these

![Figure 1](image-url)
unique changes provide insights into mechanisms associated with skeletal dyshomeostasis. To this end, we performed transcriptome profiling of primary skin fibroblasts, since S2P is involved in the RIP-regulated activation of the aforementioned transcription factors. Lipid analyses, electron microscopy and immunofluorescence assay were subsequently performed for the functional validation of the transcriptomics data.

**MATERIALS AND METHODS**

**Subjects and Cell Culture**

Punch skin biopsies were obtained from OI and IFAP/KFSD patients with pathogenic MBTPS2 variants to establish primary skin fibroblasts cultures. The biopsies of three MBTPS2-OI patients (two patients with c.1515G>C; p.(Leu505Phe) and one patient with c.1376A>G; p.(Asn459Ser)), two MBTPS2-IFAP/KFSD patients (c.1286G>A; p.(Arg429His) and c.1523A>G; p.(Asn508Ser)) and four healthy controls were used for this study (Supplementary Table 1). Informed consent of the patients or their parents, in accordance with requirements of the local ethics committees of the referring physicians, was obtained. This study was conducted according to the Declaration of Helsinki and approved by Swiss Ethics Committee (KEK-ZH-Nr. 2019-00811).

Cells were cultured at 37°C and 5% CO2 in Dulbecco’s Modified Eagle’s Medium (Gibco, 31966-021) supplemented with 10% fetal bovine serum (Gibco, 10270-106), and antibiotic-antimycotic (Gibco, 15240-06215240062) compromising of 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml Amphotericin B. Passage numbers of the cells used throughout all experiments in this study ranged between P8 and P17.

**Gene Expression Profiling**

Fibroblasts were passage into T75 flasks, fed fresh medium in the absence or presence of 500 nM thapsigargin (Sigma, T9033) 24 h after passaging, and RNA was isolated 20 h later without synchronization for transcriptome profiling. RNA was harvested using miRNeasy Mini Kit (QIAGEN, 217004) according to the manufacturer’s instructions. RNA quality control was performed on an Agilent 2100 Bioanalyzer with RNA integrity number (RIN) values between 9.6 and 10.0. Poly-A purified libraries were prepared using the TruSeq mRNA sample preparation kit (Illumina, 20020595). RNA-sequencing was performed on an Illumina HiSeq 4000 instrument at the Functional Genomics Center Zurich. The raw reads were cleaned by removing adapter sequences, trimming low quality ends, and filtering reads with low quality (phred quality < 20). Sequence alignment of the resulting high-quality reads to the human genome (build GRCh38, p10) and quantification of transcript expression was carried out using RSEM (version 1.3.0, RRID: SCR_013027) with Ensembl gene models of release 89. A count based negative binomial model implemented in the software package edgeR (R-version 3.5.1, edgeR 3.24.2, RRID: SCR_012802) was applied to detect differentially expressed genes (DEGs). Principal Component Analyses (PCA) were performed using the pcomp function in the R package “stats.” The RNA-sequencing data, including raw sequence files for each subject, have been

**FIGURE 1** Predicted secondary structure of S2P based on Uniprot topology annotations. The figure was generated using the TOPO2 display software for transmembrane proteins (Johns, 2020). The histidine residues of the His-Glu-(any amino acid)-His (H171:EXXH) motif and aspartate residue of the L466DG sequence, depicted in green, coordinate the catalytic site zinc atom of S2P. Known IFAP/KFSD-causative variants are depicted in blue; OI-causative variants are depicted in magenta.
uploaded onto the European Nucleotide Archive (ENA) under the accession ID PRJEB42767.

Differentially expressed genes were defined as those whose expression level differed between patient and control groups with \( p < 0.05 \) and \( \log_{2}(\text{fold change}) > 0.5 \) or \( < -0.5 \). This definition was used for the generation of volcano plots for visual representation of the RNA-sequencing dataset.

To identify biological processes, cellular components and molecular functions that are over-represented by DEGs in MBTPS2-OI and MBTPS2-IFAP/KFSD, genes whose expression levels differed between patient and control groups with \( p < 0.01 \) and \( \log_{2}(\text{fold change}) > 0.5 \) or \( < -0.5 \) were subjected to over-representation analysis (ORA) using the WebGestalt bioinformatics toolkit (Zhang et al., 2005; Wang et al., 2017), RRID: SCR_006786. Gene ontology (GO) terms with false discovery rate (FDR) < 10% were considered significantly enriched.

**Immunofluorescence Assay**

Fibroblasts were plated on glass cover slips and cultured in macromolecularly crowded medium containing 37.5 mg/ml Ficoll PM70 (Sigma, F2878), 25 mg/ml Ficoll PM400 (Sigma, F4375) and 0.5% fetal bovine serum (Gibco, 10270-106) and replenished daily with 50 µg/ml of freshly prepared ascorbic acid (Fluka, 95210) to accelerate the deposition of extracellular matrix proteins as previously described (Kumar et al., 2015). After 4 days, samples were fixed in 4% formaldehyde for 15 mins at room temperature and washed three times with Phosphate-Buffered Saline (PBS). Samples were incubated for 1 h at room temperature with blocking buffer containing 1% bovine serum albumin (Sigma, A3912) in PBS and subsequently incubated overnight at 4°C with antibodies against collagen type IV (Abcam, ab86042, RRID: AB_1924897). The samples were then washed with PBS 3 times, incubated for 1 h at room temperature with Alexa Fluor 594-conjugated anti-mouse IgG antibodies (Invitrogen, A11020, RRID :AB_141974), washed with PBS 3 times, and distilled water once before mounting on glass slides with 2 ml of 4% potassium hydroxide (Carl Roth GmbH, Karlsruhe, Germany) at a concentration of 1 mg/ml. The internal standard (IS), 5α-Cholestanol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), was prepared in ethanol (Carl Roth GmbH, Karlsruhe, Germany) at a concentration of 1 mg/ml. n-Hexane (Carl Roth GmbH, Karlsruhe, Germany) was used as internal standard to each sample. For sample preparation, 160–200 µl of a cell suspension corresponding to around 0.5 mg protein was used. 100 µl of 0.25 mM 5α-Cholestanol was added as internal standard to each sample. For saponification, samples were heated with 2 ml of 4% potassium hydroxide (Carl Roth GmbH, Karlsruhe, Germany) solution in ethanol at 60°C for 60 min in a heating block. The reaction mixture was diluted with 2.1 ml of purified water and acidified with 250 µl of 5N hydrochloric acid (HCl). After addition of solid sodium chloride, the samples were extracted twice with 4 ml hexane. The combined hexane fractions were dried over anhydrous sodium sulfate and then the solvent was evaporated at 60°C under a stream of nitrogen. Samples were derivatized with 50 µl N-methyl-N-(trimethylsilyl)heptafluorobutyramide (MSHFBA, Macherey-Nagel, Düren, Germany) for 1 h at 60°C and diluted with 50 µl hexane prior to injection. For GC/MS analysis, the quadrupole mass spectrometer MSD 5977A (Agilent, Santa Rosa, CA, United States) was run in the selective ion-monitoring mode with electron impact ionization. Gas chromatographic separation was achieved on a capillary column (DB-5MS, 30 m × 0.25 mm; film thickness: 0.25; J&W Scientific, Folsom,
CA, United States) using helium as carrier gas. A volume of 1 µL of the derivatized sample was injected in split mode. GC temperature parameters were 100°C for 2 mins, ramp 35°C/min to 300°C. Injector temperature was set to 280°C and interface temperature to 290°C. Quantification ions for sterols of interest were m/z 217 (5α-cholestanol), m/z 329 (cholesterol), m/z 351 (7-dehydrocholesterol), m/z 372 (desmosterol), m/z 255 (lathosterol), m/z 393 (lanosterol), and m/z 349 (7-dehydrodesmosterol).

Electron Microscopy Analysis

Fibroblasts from confluent T75 flasks were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer for 1 h at room temperature. The cells were then washed three times with 0.1 M cacodylate buffer for 5 mins per washing step to remove the fixation solution, harvested with a cell scraper, centrifuged at 13,000 rpm for 10 mins, washed three times with 0.1 M cacodylate buffer for 5 mins per washing step, post-fixed in 1% osmium tetroxide, dehydrated by a graded series of ethanol and embedded in Epon overnight at 65°C. Semi-thin and ultra-thin sections of embedded cells were prepared with an ultramicrotome and viewed with a transmission electron microscopy at the Center for Microscopy and Image Analysis, University of Zurich.

RESULTS

Transcriptome Profiling and Differential Expression Analysis

Volcano plots illustrating the statistical significance against fold change in gene expression between MBTPS2-OI and control or between MBTPS2-IFAP/KFSD and control cells cultured at basal conditions (no treatment) are depicted (Figure 2). In skin fibroblasts derived from MBTPS2-OI patients, RNA-sequencing found 841 DEGs in comparison to controls, of which 330 genes were upregulated and 511 were downregulated in the patients’ cells (Figure 2A). When fibroblasts derived from MBTPS2-IFAP/KFSD were compared to controls, 838 DEGs were identified, of which 321 were upregulated and 517 were downregulated in these patients’ skin fibroblasts (Figure 2B). 1540 DEGs were identified between MBTPS2-OI and MBTPS2-IFAP/KFSD, of which 888 were upregulated and 652 were downregulated in MBTPS2-OI compared to MBTPS2-IFAP/KFSD. The lists of DEGs in MBTPS2-OI and MBTPS2-IFAP/KFSD compared to controls can be found in Supplementary Tables 3, 4, respectively. Genes that are differentially expressed between MBTPS2-OI and MBTPS2-IFAP/KFSD are listed in Supplementary Table 5. 68 DEGs were upregulated in both MBTPS2-OI and MBTPS2-IFAP/KFSD compared to control whereas 119 DEGs were downregulated in both diseases (Supplementary Figure 1 and Supplementary Table 6). The observed numbers of overlapping DEGs are higher than what would be expected by chance based on the total number of genes identified by RNA-sequencing and the number of DEGs for each condition (Fisher’s exact test p < 0.0001).

A 5124 DEGs were identified in controls in response to thapsigargin treatment (2472 upregulated, 2652 downregulated), 4217 DEGs were identified in MBTPS2-OI in response to thapsigargin treatment (2191 upregulated, 2026 downregulated) and 5036 DEGs were identified in MBTPS2-IFAP/KFSD in response to thapsigargin treatment (2509 upregulated, 2527 downregulated). Furthermore, in response to thapsigargin treatment, 184 genes were upregulated and 213 genes were downregulated in MBTPS2-OI compared to controls, whereas 191 genes were upregulated and 171 genes were downregulated in MBTPS2-IFAP/KFSD compared to controls. 532 DEGs were observed between thapsigargin-treated MBTPS2-OI cells and MBTPS2-IFAP/KFSD cells, of which 247 were upregulated and 285 were downregulated in MBTPS2-OI.

The multivariate data analysis method principal components analysis (PCA) was performed to visualize the degree of similarities between gene expression profiles of the control and patient fibroblasts in a two-dimensional plot. Although no strong clustering of control and patient samples were observed, fibroblasts from patients with OI appear more similar to each other as did fibroblasts from IFAP/KFSD patients under basal conditions. Larger variations within the control samples are observed, where controls 1 and 4 were more similar to each other but different from controls 2 and 3. The strong effects of thapsigargin on gene expression changes are also observed, as seen by clustering of untreated samples and samples treated with thapsigargin (Supplementary Figure 2).

Pearson’s correlation coefficient was also analyzed for each pair of samples (Supplementary Figure 3). The strong effects of thapsigargin on gene expression changes are evident from the lower values of correlation coefficient obtained between no treatment (nt) and thapsigargin-treated (TG) samples. At basal conditions (nt), the IFAP and KFSD samples are more similar to each other (higher coefficient values as depicted by darker shade of gray) than to OI samples (lower coefficient values as depicted by lighter shade of gray). Likewise, OI samples are more similar to one another than to IFAP/KFSD samples. The larger variation in controls is also evident from the correlation coefficients, similar to the observations from the PCA analysis.

Next, a list of known target genes was generated through a search on the Harmonizome database (Rouillard et al., 2016) for TRANSFAC-curated transcription factor targets and a literature search for ATF6 (Jang et al., 2012; Shoulders et al., 2013; Chiang et al., 2017; Mügge and Silva, 2017; Vellanki et al., 2010; Okuda et al., 2014), BBF2H7 (Izumi et al., 2012; Kondo et al., 2018), and SREBP (Horton et al., 2003; Sharpe and Brown, 2013). The isoforms of SREBP (SREBP1a, -1c encoded by SREBF1 and SREBP2 encoded by SREBF2) were collectively analyzed since many of the target genes are transcriptionally regulated by both SREBP1 and SREBP2 (Horton et al., 2003). Heatmaps were generated to visualize the expression of these known target genes of ATF6, OASIS, BBF2H7, and SREBP in control, MBTPS2-OI and MBTPS2-IFAP/KFSD fibroblasts at basal conditions (no treatment, nt) or upon ER stress induction by thapsigargin (TG) treatment. The heatmaps are generated using the R-Function: heatmap.2 from package gplots, with the parameter Rowv = TRUE. For each gene, the colors indicate the log2 difference relative to the average of all
The order of the samples depicted in the heatmaps follow that listed in Supplementary Table 1.

The induction of ATF6-dependent genes in response to thapsigargin is comparable in control, OI and IFAP/KFSD fibroblasts (Figure 3) suggesting that the ATF6-driven ER stress response is not altered by pathogenic variants in MBTPS2. ATF6-dependent genes are also not differentially expressed between control cells and patient cells at basal conditions (Supplementary Figure 4). This is in contrast with our previous observations by overexpression of wildtype and mutant MBTPS2 constructs with p5xATF6-luciferase reporter assay (Lindert et al., 2016). Nevertheless, electron microscopy analyses of fibroblast cultures in vitro revealed the presence of abnormally shortened and enlarged ER cisterns in MBTPS2-OI and MBTPS2-IFAP/KFSD patient fibroblasts in contrast to longer and slender ER cisterns present in control fibroblasts (Figure 4), suggesting that pathogenic variants of MBTPS2 impose some form of cellular stress in both OI and IFAP/KFSD.

Additionally, we previously observed defective cleavage of OASIS in MBTPS2-OI and MBTPS2-IFAP/KFSD patient fibroblasts compared to controls (Lindert et al., 2016). However, RNA-sequencing revealed downregulation of only PAPSS2 and CHST3 expression whilst the other known OASIS-dependent genes are not differentially expressed in MBTPS2-OI and MBTPS2-IFAP/KFSD fibroblasts compared to controls, while the BBF2H7 target gene anti-apoptotic factor ATF5 is upregulated in MBTPS2-OI and MBTPS2-IFAP/KFSD fibroblasts compared to controls (Supplementary Figure 6). Upregulation of SEC23A and MIA3 in response to thapsigargin treatment was observed in patient and control cells (Figure 6). A plausible explanation for the minor differences observed in the expression of OASIS- and BBF2H7-dependent genes in S2P-defective cells is that skin fibroblasts are not physiologically relevant cell models for detecting alterations in OASIS and BBF2H7 activities, which play major roles in the development of osteoblasts in the bone and chondrocytes in the cartilage, respectively.

Interestingly, the expression of SREBP-dependent genes is suppressed in both MBTPS2-OI and MBTPS2-IFAP/KFSD patient fibroblasts compared to controls at basal condition. This effect was more pronounced in MBTPS2-OI than in MBTPS2-IFAP/KFSD patients’ cells (Figure 7). Thapsigargin treatment led to an induction of several SREBP-dependent genes in the fibroblasts except for cells derived from the MBTPS2-OI patient with the c.1376A>G (p.Asn459Ser) variant (Supplementary Figure 7). These data suggest that the activity of SREBP in activating transcription of its target genes is impaired in cells carrying pathogenic variants of MBTPS2, with a stronger impairment seen in cells with OI variants than in cells with IFAP/KFSD variants.

Gene Ontology
Given the limited availability of patient-derived cells due to the rarity of the disease and the novelty of the described pathogenic variants in MBTPS2, the sample size in our study is inevitably too small to generate meaningful FDR for the differences in gene expression between patient and control cells by RNA-sequencing. Therefore, we used a more stringent...
FIGURE 3 | Heatmap depicting expression levels of genes that are transcriptionally regulated by ATF6 at basal conditions (nt: no treatment) and in the presence of thapsigargin (TG). The dendrogram on the left y-axis shows clustering of the genes.

*p*-Value cutoff of *p* < 0.01 (instead of *p* < 0.05) and log$_2$ fold change > 0.5 or < −0.5 to generate a list of DEGs for ORA using the RNA-sequencing dataset. ORA was performed using the WebGestalt bioinformatics toolkit (Zhang et al., 2005; Wang et al., 2017) to identify biological processes, cellular components and molecular functions that are over-represented by the DEGs in MBTPS2-OI and in MBTPS2-IFAP/KFSD compared to healthy control fibroblasts.

ORA results are summarized in Table 1 for MBTPS2-OI and in Table 2 for MBTPS2-IFAP/KFSD. The analysis revealed that genes which code for extracellular matrix (ECM) proteins (GO term GO:0031012) were over-represented in both the DEGs in MBTPS2-OI and MBTPS2-IFAP/KFSD patient-derived fibroblasts in comparison to controls. Comparison of the DEGs sharing the GO term “ECM” in MBTPS2-OI and MBTPS2-IFAP/KFSD revealed that the genes CCN5, DCN and TGFB3 were upregulated in both MBTPS2-OI and MBTPS2-IFAP/KFSD compared to healthy controls whereas COL4A1, COL4A2 and GPC1 were downregulated. Furthermore, the downregulation in COL4A1 and COL4A2 gene expression translated into a reduction in deposition of collagen type IV protein in the extracellular matrix by both MBTPS2-OI and MBTPS2-IFAP/KFSD patient fibroblasts compared to controls (Figure 8).

Amongst the enriched GO terms that are listed in Tables 1, 2, several more might be potentially interesting in the understanding of the pathomechanisms and how the reviewed MBTPS2-mutations lead to two distinct phenotypes. These include skin development (GO:0043588), epidermis development...
Lim et al. Omics Profiling of MBTPS2-Osteogenesis Imperfecta

FIGURE 4 | In vitro electron microscopy analyses of fibroblasts derived from (A,B) healthy controls, (C–E) MBTPS2-OI and (F,G) MBTPS2-IFAP/KFSD patients. In each image a portion of the fibroblasts is depicted with abundant ER cisterns. Short and large shaped ER cisterns are shown in the MBTPS2-OI [(C–E), white arrows] and MBTPS2-IFAP/KFSD patients [(F,G), white arrows] in contrast to the long and slender shaped ER cisterns shown in the two controls [(A,B), white large arrows].

(GO:0008544), cell-substrate adhesion (GO:0031589), collagen trimer (GO:0005581), receptor complex (GO:0043235) and ECM-binding (GO:0050840).

qRT-PCR

Based on the above analyses suggesting impaired SREBP activity in MBTPS2-mutant cells, we selected several SREBP-dependent genes involved in fatty acid metabolism (SCD, FADS1, FADS2, and ACSL3) and sterol metabolism (FDFT1, EBP, DHCR7, and DHCR24) for validation by qRT-PCR; OASIS-dependent genes involved in skeletal development (CHST3 and PAPSS2) were also selected. Furthermore, in the top hits of DEGs in MBTPS2-OI compared to controls we identified several genes involved in processes concerning either bone or cartilage homeostasis, which could contribute to the bone phenotype observed in OI patients. These genes, namely VEGFA, ADAMTS12 and DKK1 were also selected for validation by qRT-PCR (Figure 9).

By qRT-PCR, we observed a significant downregulation in the expression of SCD, FADS1, FADS2 and ACSL3 in MBTPS2-OI patient fibroblasts, and a trend towards suppression of FADS1 and FADS2 expression in MBTPS2-IFAP/KFSD patient fibroblasts (Figure 9A), in line with the RNA-sequencing data. A suppression of ACSL3 expression was also observed...
in MBTPS2-IFAP/KFSD but to a smaller magnitude to that seen in MBTPS2-OI (Figure 9A), although this suppression was not significant by RNA-sequencing. Since these genes are involved in the synthesis of fatty acid, the data suggests perturbations in fatty acid metabolism in response to pathogenic MBTPS2 variants, likely with a stronger effect in MBTPS2-OI...
than in MBTPS2-IFAP/KFSD. Therefore, fatty acid content was subsequently measured in cultured fibroblasts for functional validation of the transcriptomics results (see section “Fatty Acid Profile of Cells”).

In line with the RNA-sequencing data, the downregulation in the expression of DHCR7 in both MBTPS2-OI and MBTPS2-IFAP/KFSD was confirmed by qRT-PCR; suppression of FDFT1, EBP and DHCR24 in MBTPS2-OI compared to controls was also demonstrated (Figure 9B). In contrary to the RNA-sequencing data which showed only modest, non-significant suppression of DHCR24 in MBTPS2-IFAP/KFSD, qRT-PCR revealed a significant downregulation of DHCR24 in MBTPS2-IFAP/KFSD fibroblasts, albeit at a smaller fold change than that seen in MBTPS2-OI cells. Collectively, the dysregulation of SREBP-dependent genes involved in the cholesterol synthesis pathways point towards perturbed sterol metabolism caused by pathogenic mutations in MBTPS2. Thus, the sterol profile of these fibroblasts was subsequently determined (see section “Sterol Profile of Cells”).

The downregulation of CHST3 in both MBTPS2-OI and MBTPS2-IFAP/KFSD and of PAPSS2 in MBTPS2-OI was confirmed by qRT-PCR (Figure 9C). A significant suppression of PAPSS2 expression was also observed in MBTPS2-IFAP/KFSD by qRT-PCR (Figure 9C), although this trend was not statistically significant ($p = 0.0677$) by RNA-sequencing.

Finally, the strong upregulation of VEGFA and ADAMTS12 and downregulation of DKK1 in MBTPS2-OI compared to both control and MBTPS2-IFAP/KFSD observed by RNA-sequencing was reproduced by qRT-PCR (Figure 9D).

**Fatty Acid Profile of Cells**

The biosynthesis of non-essential fatty acids is catalyzed in sequential steps by many enzymes whose expression is regulated by SREBP at the transcript level, as depicted in a simplified
The ratio of oleic acid to stearic acid is decreased in MBTPS2-OI fibroblasts compared to controls (Figure 11A), which demonstrates reduced stearoyl-CoA desaturase activity that converts stearic acid to oleic acid. This is consistent with the suppressed gene expression of SCD encoding stearoyl-CoA desaturase.

The diagram in Figure 10. Therefore, we quantified the cellular content of several of these fatty acids in control and patient fibroblasts by GC-MS/MS for the functional validation of the suppressed expression of SREBP target genes involved in fatty acid metabolism.
desaturase in MBTPS2-OI patient fibroblasts but not MBTPS2-IFAP patient fibroblasts (Figure 9A).

FADS1 and FADS2 encode the Δ⁵-desaturase and Δ⁶-desaturase enzymes, respectively, and are involved in the biosynthesis of long chain omega-6 fatty acids from linoleic acid and long chain omega-3 fatty acids from α-linolenic acid (Figure 10). In particular, the Δ⁵-desaturase enzyme is the rate-limiting enzyme in the metabolism of linoleic acid to arachidonic acid and of α-linolenic acid to docosapentaenoic acid (Horrobin et al., 1993; Cho et al., 1999). Since the gene expression of FADS1 and FADS2 is suppressed in MBTPS2-OI fibroblasts compared to controls (Figure 9A), we hypothesized alterations in the metabolism of omega-3 and omega-6 fatty acids in these cells.

For omega-6 fatty acids, the ratios of γ-linolenic acid to linoleic acid (GLA / LA), arachidonic acid to di-homo-γ-linolenic acid (AA / DGLA), and arachidonic acid to linoleic acid (AA / LA) are not significantly altered (Figure 11B) despite FADS2 and FADS1 suppression.

For omega-3 fatty acids, the ratio of eicosapentaenoic acid to α-linolenic acid (EPA / ALA) is not significantly altered in MBTPS2-OI fibroblasts (Figure 11C). However, in MBTPS2-OI fibroblasts, a significant reduction in the ratio of docosahexaenoic acid to docosapentaenoic acid (DHA / DPA) is observed (Figure 11C), consistent with the suppression of FADS2, which can convert DPA to DHA directly through its Δ⁴-desaturase activity or via tetracosapentaenoic acid and tetracosahexaenoic acid with its Δ⁶-desaturase activity. On the other hand, the ratio of docosapentaenoic acid to eicosapentaenoic acid (DPA / EPA) is significantly increased in MBTPS2-OI fibroblasts (Figure 11C). This elevated ratio is not surprising since the reduction in FADS2...
and FADS1 expressions can block the synthesis of EPA from ALA and at the same time lead to an accumulation in DPA due to a block in the desaturation of DPA to DHA.

**Sterol Profile of Cells**

The biosynthesis of cholesterol is a multi-step process catalyzed by several enzymes whose expression is transcriptionally regulated by SREBP. The first few steps require the conversion of acetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and then to mevalonate. Mevalonate undergoes phosphorylation, decarboxylation and condensation to form lanosterol, which is the first sterol synthesized in this pathway. Subsequently, the conversion of lanosterol to cholesterol occurs via the Bloch pathway (involving the sterol intermediates 7-dehydrodesmossterol and desmossterol) or the Kandutsch-Russell pathway (involving the sterol intermediates lahydrosterol and 7-dehydrocholesterol), or a hybrid of both pathways, catalyzed by multiple enzymes including EBP, DHCR7 and DHCR24 (Kandutsch and Russell, 1960; Bloch, 1965; Mitsche et al., 2015). Since RNA-sequencing and qRT-PCR demonstrated the downregulation of EBP, DHCR7 and DHCR24 in MBTPS2-mutant cells, we attempted to quantify the cellular content of the sterol metabolites in control and patient fibroblasts. In consideration of previous findings in which cells lacking endogenous Mbtps2 do not survive in cholesterol-deficient conditions and overexpression of wildtype but not mutant
MBTPS2 rescues this phenotype (Oeffner et al., 2009), we cultured our fibroblasts in standard sterol-replete medium. Intracellular levels of lanosterol, 7-dehydrodesmosterol, desmosterol, lathosterol, 7-dehydrocholesterol and cholesterol were measured by GC-MS/MS; ratios of these sterol metabolites were calculated to assess the conversion of one metabolite to another. Unfortunately, we could not detect consistent alterations in the sterol profiles of the control and patient fibroblasts (Supplementary Figure 8), likely due to the presence of sterols in the culture medium that can cause feedback inhibition of HMG-CoA reductase thus resulting in a reduction in endogenous cholesterol biosynthesis. Therefore, the effects of MBTPS2 mutations in OI and IFAP/KFSD on sterol metabolism and cholesterol synthesis remains to be better characterized.

**DISCUSSION**

MBTPS2 mutations have been identified as the first X-linked cause of OI that is associated with a moderate to severe, potentially intrauterine lethal clinical phenotype (Lindert et al., 2016). Peculiarity, MBTPS2 pathogenic variants distinct from those identified in OI were already known to cause the dermatological spectrum condition IFAP and KFSD (Oeffner et al., 2009; Aten et al., 2010; Bornholdt et al., 2013). This offers a unique biological context to identify molecular players and mechanisms that are specifically perturbed in MBTPS2-OI but not in MBTPS2-IFAP/KFSD and are thus relevant to the pathophysiology in the skeletal system.

Through transcriptome profiling of fibroblasts obtained from OI patients, IFAP/KFSD patients and healthy donors, we identified unique gene expression changes in OI cells; these genes play biological roles in bone and cartilage development, or regulate lipid metabolism. Furthermore, we found that the relative abundance of fatty acids in OI fibroblasts were altered. These in vitro findings generate new insights on biological molecules and pathways that could be relevant to the progression of OI and open up new hypotheses on the pathomechanisms underlying OI that should be further tested in more relevant in vitro and in vivo models of bone and cartilage. Importantly, this will pave the way towards the development of OI-centric therapeutic strategies.

In the remaining discussion section of the manuscript, we highlight the biological functions of genes that are differentially expressed in MBTPS2-OI and their potential contribution to the pathogenesis of OI.
**SREBP-Dependent Genes**

*EBP* (Emopamil-binding protein, also known as EBP cholestenol delta-isomerase) is significantly downregulated in *MBTPS2-OI* patient fibroblasts (Figure 9B and Supplementary Table 3) and displays a trend towards downregulation in *MBTPS2-IFAP/KFSD* patient fibroblasts (p = 0.0660, Figure 9B). EBP cholestenol delta-isomerase catalyzes the conversion of Δ⁸-sterols to their corresponding Δ⁷-isomers in cholesterol biosynthesis. Loss-of-function mutations in *EBP* on the X-chromosome have been previously identified in Conradi-Hünermann-Happle-syndrome, which is also known as X-linked dominant chondrodysplasia punctata 2 (CDPX2) (OMIM #302960). Clinical manifestations of CDPX2 include short stature, skeletal abnormalities consisting of rhizomelic shortening of the limbs, stippling and scoliosis, skin abnormalities including scaling ichthyosis and coarse scalp hair with scarring alopecia (Kumble and Savarirayan, 2020). Strikingly, these clinical features appear to partially overlap with that of *MBTPS2-OI* patients in terms of short stature and skeletal abnormalities, as well as with that of IFAP and KFSD patients with skin abnormalities. Therefore, deficiency in EBP cholestenol delta-isomerase affecting sterol metabolism seems to affect multiple tissues.

*ACSL3* encodes the Long-chain-fatty-acid-CoA ligase 3 which catalyzes the production of long chain fatty acyl-CoA esters from free long-chain fatty acids, which then serve as a substrate for lipid synthesis and β-oxidation. Thus, a downregulation of *ACSL3* observed in *MBTPS2* mutant cells (Figure 9A) may alter...
cellular metabolism, energy production and cell survival, similar to that observed in lung cancer cells (Padanad et al., 2016). The involvement of ACSL3 in inducing osteoblastic differentiation of vascular smooth muscle cells has also been demonstrated as one of the pathophysiological processes in the development of vascular calcification. Inhibition of ACSL3 blocked palmitic acid-induced osteoblastic differentiation of vascular smooth muscle cells as evident from attenuated expression of BMP2 and MSX2 (Kageyama et al., 2013). Furthermore, in a study performed in a cohort of 91 females, ACSL3 was found to be correlated to bone mineral density (Reppe et al., 2010). These suggest that ACSL3 plays a role in maintaining bone health and development, although the mechanism(s) remains to be elucidated.

Our data also demonstrated reduced expression of several SREBP-dependent genes involved in fatty acid metabolism and changes in fatty acid relative abundance in MBTPS2-OI cells. This is an interesting finding since numerous studies have shown an association between fatty acid and bone health (reviewed by Salari et al., 2008; Mangano et al., 2013; Wauquier et al., 2015). A high dietary intake of the monounsaturated fatty acid oleic acid positively regulates calcium absorption, bone mineralization and bone density in mice (Rezq et al., 2010) and prevents ovariectomy-induced osteoporosis in rats (Saleh and Saleh, 2011). In humans, a positive association between high oleic acid intake through the consumption of olive oil and bone mineral density has also been observed (Trichopoulou et al., 1997; Roncero-Martín et al., 2018). For polyunsaturated fatty acids (PUFA), cross-sectional and longitudinal studies in various populations have led to variable observations (Mangano et al., 2013). However, more recent randomized control trials suggest improved bone status with long-chain PUFA supplementation (Martin-Bautista et al., 2010; Lappe et al., 2013; Mangano et al., 2013). A 6-month intervention with genistein, vitamin D3, vitamin K1 and PUFA (EPA and DHA) improved the bone mineral density in post-menopausal women (Lappe et al., 2013). In another study, a 12-month intervention with EPA, DHA, oleic acid, and several vitamins including vitamin D led to an improvement in the patients serum of the bone formation biomarkers osteoprotegerin (OPG), receptor activator of nuclear factor κB ligand (RANKL), OPG/ RANKL ratio and osteocalcin (Martin-Bautista et al., 2010). However, in these studies, the multiple component nature of the interventions hinders a sound conclusion on the specific effects of long chain PUFA on bone health. In animal studies, PUFA supplementation prevents the loss of bone weight and strength caused by estrogen deficiency or calcium deficiency (Sakaguchi et al., 1994) while a diet rich in DHA lead to higher bone mineral density and bone mineral content (Lukas et al., 2011) in rats. Long term dietary intake of EPA also improved the structural and mechanical properties of cortical bone in mice (Bonnet and Ferrari, 2011), while dietary long chain omega-3 PUFA’s were protective against age-induced bone loss in rats (Shen et al., 2006). The mechanisms underlying the effects of these fatty acids on bone health has also been investigated (reviewed in Wauquier et al., 2015). For instance, omega-3 PUFA’s can promote osteoblast differentiation via increased production of insulin-like growth factor-1 and parathyroid hormone (Shen et al., 2006). Furthermore, lipids are required to form vesicles for transporting vitamins D and K, which are involved in regulating serum calcium and phosphorous concentrations and activation of osteocalcin for bone mineralization (Tintut and Demer, 2014). Therefore, the significant suppression of SCD, FADS1 and FADS2 specifically in MBTPS2-OI patient fibroblasts and the resultant hindered conversion of stearic acid to oleic acid, ALA to EPA and DPA to DHA may negatively affect bone metabolism and health. In a recent study (Wang et al., 2020), monoallelic pathogenic variants in SREBF1 encoding for the SREBP1 transcription factor were found to cause IFAP in individuals who do not carry pathogenic variants of MBTPS2. The SREBF1 mutations lead to impaired cleavage of SREBP1 by S1P, thereby hindering the translocation of SREBP1 to the nucleus. RNA-sequencing of skin from the scalp of affected individuals revealed the downregulation of genes involved in lipid metabolism, although the serum lipid profiles of the patients appeared normal. Of particular interest to us, the downregulation of SCD in the SREBF1-IFAP patients match our findings of downregulation of SCD in the MBTPS2-OI fibroblasts. However, SCD was not downregulated in MBTPS2-IFAP/KFSD fibroblasts. Despite significant downregulation of SCD with consequent alterations in the relative abundance of fatty acids in MBTPS2-OI fibroblasts, the MBTPS2-OI patients in our study did not present with alopecia or photophobia.

### OASIS-Dependent Genes

In the list of OASIS-dependent genes, CHST3 and PAPSS2 were significantly downregulated in both MBTPS2-OI and MBTPS2-IFAP/KFSD.

**CHST3** encodes chondroitin 6-O-sulfotransferase (C6ST-1), an enzyme that catalyzes the sulfation of chondroitin. Loss-of-function mutations in **CHST3** are causative of spondyloepiphyseal dysplasia (SED) Omani type (OMIM 143095) (Thiele et al., 2004), in which a missense mutation in **CHST3** leads to a generalized defect in the chain sulfation of chondroitin sulfate resulting in severe chondrodysplasia. Affected patients display normal length at birth but severely reduced height at adulthood, progressive kyphoscoliosis and joint dislocations leading to restricted joint movement (Thiele et al., 2004). Similar to the SED Omani type patients, MBTPS2-OI patients also have a short stature and both patients from the German pedigree presented with kyphoscoliosis.

**PAPSS2** encodes 3′-phosphoadenosine 5′-phosphosulfate (PAPS) synthase 2 which synthesizes PAPS, a universal donor of sulfate required for the sulfation of many glycosaminoglycans, proteins and steroid hormones. Specifically, PAPS is required for the sulfation of the glycosaminoglycan chondroitin sulfate, which is an important component of cartilage. Loss-of-function mutations in **PAPSS2** are associated with autosomal-recessive forms of brachydactyly, a heterogeneous group of skeletal dysplasias, including the Hobaek (OMIM 271530) and Toledo (OMIM 271630) types (Miyake et al., 2012; Iida et al., 2013). Affected individuals show short-trunk short stature, scoliosis and broad proximal interphalangeal joints. In our study, **PAPSS2** was significantly downregulated in the fibroblasts of both MBTPS2-OI and MBTPS2-IFAP/KFSD patients.
(Figure 9C), which might contribute to the two phenotypes in a yet unknown way.

**BBF2H7-Dependent Genes**

Regulated intramembrane proteolysis involving S1P and S2P encoded by MBTPS1 and MBTPS2, respectively, has been previously shown to be an important process in chondrogenesis. This is mediated by RIP activation of the BBF2H7 transcription factor, which in turn upregulates the expression of SEC23A (Saito et al., 2009), SERPINH1, MIA3 and TRAPPC2 (Kondo et al., 2018) essential for the formation of mega vesicles for ECM protein secretion in cartilage development. Schombs et al. (2003) observed that knockdown of either Mbp1n or Mbp2t leads to cartilage defects in zebrafish as demonstrated by reduced Alcian blue staining. S1P deficiency caused by a mutation in MBTPS1 was recently described in a pediatric patient with retarded growth and skeletal dysplasia presenting with reduced bone mineral density, spondyloepiphyseal dysplasia, kyphosis and dysmorphic facial features (Kondo et al., 2018). In this patient, the expression of BBF2H7-dependent genes SEC23A, SERPINH1, MIA3, and TRAPPC2 crucial for ECM protein secretion was reduced, coupled with abnormal intracellular retention of collagen and enlarged ER (Kondo et al., 2018). Similarly, deletion of Bbf2h7 in mice led to severe chondrodysplasia, abnormal expansion of the ER containing aggregated type II collagen and cartilage oligomeric matrix protein (COMP) and death by suffocation shortly after birth due to an immature chest cavity (Saito et al., 2009). Such defective chest cavity formation was also observed in our cohort of MBTPS2-OI patients in the form of pectus excavatum and pectus carinatum (Lindert et al., 2016). Abnormal, rounded ER morphologies were also observed in MBTPS2-OI and MBTPS2-IFAP/KFSD patient fibroblasts in vitro by electron microscopy analyses (Figure 4), although our transcriptomics analysis of patient-derived fibroblasts revealed only subtle differences in the expression of BBF2H7-dependent genes (Figure 5). In addition, an alternative mechanism of BBF2H7-regulated chondrogenesis has been proposed, in which the C-terminus fragment of BBF2H7 is secreted into the extracellular space upon RIP cleavage and binds directly to Indian hedgehog and its receptor Patched-1 to activate Hedgehog signaling and promote proliferation of chondrocytes (Saito et al., 2014). Therefore, it would be interesting to further investigate whether the pathogenic MBTPS2 variants associated with either OI or IFAP/KFSD have different effects on BBF2H7 transcriptional activities and Hedgehog signaling causing impaired chondrogenesis in in vitro and ex vivo models of cartilage.

**Other Genes and Pathways Involved in Skeletal Development**

Untargeted transcriptome analysis has delivered further evidence pointing towards perturbed skeletal development in MBTPS2-OI. Specifically, Vascular Endothelial Growth Factor A (VEGFA) and A Disintegrin And Metalloproteinase with Thrombospondin type 1 motif 12 (ADAMTS12) are strongly upregulated in MBTPS2-OI fibroblasts (Figure 9D and Supplementary Table 3).

Widely known for its role in angiogenesis, VEGFA (also known as VEGF before the discovery of other VEGF family members) is also involved in regulating growth plate morphogenesis, cartilage remodeling and bone formation (Gerber et al., 1999). The upregulation of VEGFA seen specifically in MBTPS2-OI could thus be associated with disturbed chondrocyte and bone development.

ADAMTS12 encodes a zinc metalloproteinase which degrades ECM components. It is strongly expressed in growth plate cartilage and has a potential inhibitory effect on chondrocyte differentiation, highlighting its involvement in chondrogenesis (Bai et al., 2009). Pathologically, the upregulation of ADAMTS12 expression and cleavage of COMP by ADAMTS12 has been demonstrated in arthritis (Luan et al., 2008; Liu, 2009). ADAMTS12 is also overexpressed in endplate cartilage of patients with degenerative disc changes, coupled with a downregulation of several marker genes for chondrogenesis (Zhang et al., 2012b). Thus, its upregulation in MBTPS2-OI patient cells suggest its potential pathogenic involvement in skeletal development.

Hence, the determination of VEGFA and ADAMTS12 levels particularly in chondrocytes of MBTPS2-OI and MBTPS2-IFAP/KFSD patients would be highly valuable for understanding their involvement in the pathomechanisms related to the skeletal phenotype seen in OI. However, the mechanisms that contribute to elevated expression of VEGFA and ADAMTS12 as a consequence of MBTPS2 mutations in OI but not in IFAP/KFSD remains to be deciphered.

DKK1 encodes the Dickkopf-1 protein, a secreted protein that antagonizes the Wnt/β-catenin signaling by binding and inactivating the lipoprotein-related protein LRP5/6 coreceptor. DKK1 is transcriptionally regulated by several factors, including the Mxs2 transcription factor highly expressed in osteoblasts, the BMP/c-Jun signaling pathway and via a negative feedback loop involving TCF/β-catenin (Grotewold and Rüther, 2002; Niida et al., 2004; Chamorro et al., 2005; Pinzone et al., 2009). DKK1 plays an important role in development; it promotes embryonic head induction and programmed cell death in the developing limb (Mukhopadhay et al., 2001; Grotewold and Rüther, 2002) and also switches the mesenchymal stem cell differentiation pathway to favor adipogenesis over osteoblastogenesis and chondrogenesis thereby preventing endochondral bone formation (Christodoulides et al., 2006; Chen et al., 2007; Pinzone et al., 2009). In addition to inhibiting osteoblast differentiation and bone formation, elevated levels of DKK1 also promote bone resorption by inducing osteoclastogenesis via increased receptor activator of NF-kB ligand (RANKL) and decreased osteoprotegerin (OPG) production (Pinzone et al., 2009). Our transcriptomics analysis revealed a downregulation in the expression of DKK1 in MBTPS2-OI patient fibroblasts (Figure 9D) suggesting that bone homeostasis may be perturbed, although its exact involvement and role in the pathomechanisms of OI requires more in-depth studies. In a recent transcriptomics study performed on whole blood of OI patients with COL1A1 mutations (Zhytnik et al., 2020), the authors observed a decreased expression of DKK3 from the same Dickkopf family of Wnt signaling antagonisers in COL1A1-OI patients compared to healthy controls. Therefore, further investigations in whether
a reduction in Wnt signaling inhibition represents a common compensatory mechanism to avoid an impairment of bone formation in OI would be very interesting.

**Genes Encoding ECM Proteins**

Genes encoding extracellular matrix (ECM) proteins (GO term GO:0031012) were found to be over-represented by ORA analysis of differentially expressed genes. COL4A1, COL4A2, and GPC1 were downregulated whereas CCN5, DCN, and TGFBR3 were upregulated in both MBTPS2-OI and MBTPS2-IFAP/KFSD compared to healthy controls.

**Downregulated Genes COL4A1, COL4A2, and GPC1**

The α1α2 heterotrimers of type IV collagen encoded by COL4A1 and COL4A2 constitute one of the most abundant components of nearly all basement membranes. After assembly, extensive post-translational modification and secretion into the ECM, they polymerize into a network and interact with other extracellular and membrane bound molecules. Mutations in COL4A1 or COL4A2 are pleiotropic and contribute to a spectrum of multi-system disorders including cerebrovascular, ocular, cerebral, renal and muscular defects (Kuo et al., 2012). COL4A1 and COL4A2 expression is downregulated by a family of microRNAs. In particular, miR-214-5p has been shown to suppress bone formation, and its inhibition in mouse osteoblastic cells MC3T3-E1 increases COL4A1 and COL1A1 expression and leads to increased cell viability. Similarly, the overexpression of COL4A1 by plasmid transfection increases cell viability and COL1A1 expression (Li et al., 2017). In line with this, we previously observed a reduction in the secretion rate of collagen type I protein by MBTPS2-OI and MBTPS2-IFAP/KFSD fibroblasts (Lindert et al., 2016). Therefore, the downregulation of COL4A1 and COL4A2 might negatively impact cell viability, osteoblast differentiation and general extracellular matrix properties of the bone in MBTPS2-OI.

GPC1 encodes glypican-1, a cell surface anchored heparan sulfate proteoglycan. Its heparan sulfate side chain mediates binding to cytokines and growth factors thus facilitating the initiation and perpetuation of cell signaling (Matsumo and Kimura-Yoshida, 2013; Wang et al., 2019). Glypican-1 has been shown to promote the interaction between fibroblast growth factor 2 (FGF2) and its receptor FGFR1, thereby stimulating FGF2 signaling (Zhang et al., 2001). Since FGF2-knockout mice have reduced bone mass and bone formation (Montero et al., 2000), we speculate that a downregulation of GPC1 may affect bone development by interfering with FGF2 signaling. Furthermore, glypican-1 has also been demonstrated to bind bone morphogenetic protein 2 (BMP2) and inhibit its functional activity in a human cranial osteogenesis model (Dwivedi et al., 2013). Therefore, a reduction in GPC1 expression could affect bone formation by altering BMP2 signaling activities.

**Upregulated Genes CCN5, TGFBR3, and DCN**

CCN5, also known as WNT1 inducible signaling pathway protein 2 (WISP-2) or Connective Tissue Growth Factor-Like Protein (CTGF-L), is a member of the connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. The CCN family of proteins play an important role during embryonic development, wound healing, injury repair, angiogenesis and fibrosis (Grünberg et al., 2018). CCN5 is a repressor of the transforming growth factor β (TGF-β) signaling pathway by restricting transcription of the TGF-βRII gene (Sabbah et al., 2011) thereby exerting anti-fibrotic effects. In addition, CCN5 induces canonical WNT pathway which is important for the determination of the fate of mesenchymal stem cells (MSC) (Grünberg et al., 2018). CCN5 is expressed in primary human osteoblast cultures (Kumar et al., 1999) and in mature murine osteocytes (Kawaki et al., 2011). Supplementation of recombinant CCN5 protein improved osteoblast mineralization and upregulated the expression of osteogenic genes osterix, alkaline phosphatase and bone sialoprotein in vitro (Kawaki et al., 2011). In addition, the gene expression of CCN5 increases during terminal differentiation of chondrocytes (Kawaki et al., 2008). These collectively suggest an anabolic effect of CCN5 on bone formation.

**TGFBR3** encodes the TGF-β type III receptor, also known as betaglycan. Although betaglycan lacks signaling capacity, it acts as a coreceptor and potentiates the binding of TGF-β ligands to the TGF-β type II receptor, therefore enhancing the TGF-β signaling pathway (Villarreal et al., 2016). Betaglycan is required for inducing osteoblast differentiation and promoting osteogenesis (Hill et al., 2015; Cook et al., 2019).

Therefore, the upregulation of CCN5 and TGFBR3 may be counterintuitive but perhaps reflect a feedback mechanism and attempt of MBTPS2-OI cells to boost bone health.

Decorin, encoded by DCN, is a proteoglycan representing a common component of connective tissue and extracellular matrix. It is composed of a protein core with a single covalently attached glycosaminoglycan (GAG) chain consisting of either chondroitin sulfate in mineralized matrices of bone and dentine or dermatan sulfate in soft connective tissues such as skin and ligament (Waddington et al., 2003). Decorin interacts with collagen fibers with high affinity and regulates collagen fibrillogenesis, thereby playing a crucial role in maintaining the structural and biomechanical properties of the connective tissue (Danielson et al., 1997; Keene et al., 2000; Zhang et al., 2006). Hence, changes in decorin expression may have an impact on bone strength and function.

**CONCLUSION**

Few OASIS-dependent genes are suppressed in MBTPS2-OI, while BBF2H7- and ATF6-dependent genes are comparable between OI and IFAP/KFSD patients and control fibroblasts. Importantly, we identified genes involved in cartilage physiology that are differentially expressed in MBTPS2-OI but not in MBTPS2-IFAP/KFSD fibroblasts.

In this study, we identified gene expression changes in response to pathogenic variants in MBTPS2 causing either OI or IFAP/KFSD through RNA-sequencing-based transcriptome profiling of fibroblasts obtained from patients and healthy
donors. The downregulation of SREBP-dependent genes is stronger in OI than in IFAP/KFSD compared to controls, with consistent changes in the relative abundance of fatty acids in MBTPS2-OI fibroblasts in vitro. Furthermore, in MBTPS2-OI fibroblasts we identified changes in the expression of genes involved in bone and cartilage development, namely CHST3 and PAPSS2 that are regulated by OASIS, and VEGFA, ADAMTS12 and DKK1. These in vitro findings generate new insights and open up new hypotheses on mechanisms that could contribute to and/or counter act the disease progression in OI. It is therefore important to promote OMICS studies in other genetic forms of OI in order to facilitate the discovery of the manifold players in the disease pathology that could be part of a common pathomechanism of OI, such as the Dickkopf family of Wnt signaling antagonisers. In a near future, the establishment of relevant in vitro and in vivo models of bone and cartilage will allow us to test these new hypotheses in order to develop OI-centric therapeutic strategies.

Study Limitations
The main limitations of our study arise from the rarity of the genetic disorders caused by MBTPS2 pathogenic variants and the difficulty in obtaining the relevant biological samples for molecular characterization. In this study the number of biological replicates available for MBTPS2-OI and IFAP/KFSD was small and only primary fibroblasts could be obtained from minimally invasive skin punch biopsies. This has limited the ability to detect alterations in physiological processes of bone and cartilage that affect the development of osteoblasts and chondrocytes, such as those regulated by the S2P substrates OASIS and BBF2H7 as discussed above.

A further layer of complexity arises from the wide range of age and variability in the genetic background of our cohort of patients. Therefore, this study has adopted a p-Value of < 0.01 without correction for multiple testing in order to discover differentially expressed genes with biologically meaningful functions that may contribute to the disease pathogenesis of OI. Nevertheless, the data obtained has allowed us to generate new hypotheses that we will test in relevant disease models in the future.

DATA AVAILABILITY STATEMENT
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/ena, PRJEB42767.

REFERENCES
Aten, E., Brasz, L. C., Bornholdt, D., Hooijkaas, I. B., Porteous, M. E., Sybert, V. P., et al. (2010). Keratosis Follicularis Spinulosa Decalvans is caused by mutations in MBTPS2. Hum. Mutat. 31, 1125–1133. doi: 10.1002/humu.21335
Bai, X. H., Wang, D. W., Luan, Y., Yu, X. P., and Liu, C. J. (2009). Regulation of chondrocyte differentiation by ADAMTS-12 metalloproteinase depends on its enzymatic activity. Cell. Mol. Life Sci. 66, 667–680. doi: 10.1007/s00018-008-8633-x
Bloch, K. (1965). The biological synthesis of cholesterol. Science (80.) 150, 19–28. doi: 10.1126/science.150.3692.19
Bonnet, N., and Ferrari, S. L. (2011). Effects of long-term supplementation with omega-3 fatty acids on longitudinal changes in bone mass and microstructure in mice. J. Nutr. Biochem. 22, 665–672. doi: 10.1016/j.jnutbio.2010.05.006

ETHICS STATEMENT
The studies involving human participants were reviewed and approved by the Swiss Ethics Committee (KEK-ZH-Nr. 2019-00811). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS
CG, MR, UL, LO, and PJL contributed to the conceptualization of the project. SM, PJL, UL, and LO contributed to transcriptomics data analyses and validation. TN and PJL contributed to immunofluorescence and electron microscopy analyses. PS, MP, and MH contributed to fatty acid analyses. C-DL and DH contributed to sterol analyses. SM, PJL, CG, LO, MH, and C-DL contributed to the manuscript preparation. All authors contributed to the review of the manuscript.

FUNDING
This work has been supported by the Swiss National Science Foundation Grant No. 31003A-173183 to CG and MR, the Gottfried and Julia Bangerter-Rhyner-Foundation, the Olga Mayenfisch Foundation, and the Uniscientia Foundation.

ACKNOWLEDGMENTS
IFAP patient material were kindly provided by Cynthia J. Tiff (Pediatric Undiagnosed Diseases Program, NHGRI, NIH, United States). KFSD patient material were kindly provided by Emmelien Aten (Department of Human and Clinical Genetics, LUMC, Leiden, Netherlands). OI patients material were kindly provided by Joan C. Marini (Section on Heritable Disorders of Bone and Extracellular Matrix, NICHD, NIH, United States), Vorasuk Shotelersuk (Center of Excellence for Medical Genetics, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Thailand), and Ingo Kennerknecht (Institute of Human Genetics, Westfalishe Wilhelms University, Germany).

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.662751/full#supplementary-material
Bornholdt, D., Atkinson, T. P., Boudjabar, B., Catteau, B., Cox, H., De Silva, D., et al. (2013). Genotype-phenotype correlations emerging from the identification of missense mutations in MBTPS2. Hum. Mutat. 34, 587–594. doi: 10.1002/humu.22275

Chamorro, M. N., Schwartz, D. R., Bonica, A., Brivanlou, A. H., Cho, K. R., and Varmus, H. E. (2005). FGF-20 and DKK1 are transcriptional targets of β-catenin and FGF-20 is implicated in cancer development and evolution. EMBO J. 24, 73–84. doi: 10.1038/sj.emboj.7600460

Chen, Y., Whetstone, H. C., Youn, A., Nadesan, P., Chow, E. C. Y., Lin, A. C., et al. (2007). β-catenin signaling pathway is crucial for bone morphogenetic protein 2 to induce new bone formation. J. Biol. Chem. 282, 526–533. doi: 10.1074/jbc.M607200200

Chiang, W. C., Chan, P., Wissinger, B., Vincent, A., Skorczyk-Werner, A., Krawczynski, M. R., et al. (2017). Achromatoplasia mutations target sequential steps of ATP6 activation. Proc. Natl. Acad. Sci. U.S.A. 114, 400–405. doi: 10.1073/pnas.1606387114

Cho, H. P., Nakamura, M. T., and Clarke, S. D. (1999). Cloning, expression, and missense mutations in MBTPS2. Hum. Mutat. 13, 471–477. doi: 10.1002/(SICI)1098-1501(199906)13:4<471::AID-HUMU10>3.0.CO;2-I

Christodoulides, C., Laudes, M., Cawthorn, W. P., Schinner, S., Soos, M., O’Rahilly, S., et al. (2006). The Wnt antagonist Dickkopf-1 and its receptors are coordinately regulated during early human adiogenesis. J. Cell Sci. 119, 2613–2620. doi: 10.1242/jcs.02975

Cook, L. M., Frielings, J. S., Nerlakani, N., McGuire, J. J., Stewart, P. A., Burger, K. L., et al. (2019). Betaglycan drives the mesenchymal stromal cell osteogenic program and prostate cancer-induced osteogenesis. Oncogene 38, 6959–6969. doi: 10.1038/s41561-019-0913-4

Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Izoz, R. V. (1997). Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J. Cell Biol. 136, 729–743. doi: 10.1083/jcb.136.3.729

DiMeglio, L. A., Ford, L., McClintock, C., and Peacock, M. (2005). A comparison of oral and intravenous bisphosphonate therapy for children with osteogenesis imperfecta. J. Pediatr. Endocrinol. Metab. 18, 43–53. doi: 10.1515/JPEM.2005.1.8.1.43

Dvela-Levitt, M., Kost-Alimova, M., Emani, M., Kohnert, E., Thompson, R., et al. (2013). Regulation of bone morphogenetic protein signalling and cranial microenvironments accelerate the development of osteogenesis imperfecta. Am. J. Med. Genet. A 161, 22275–2237. doi: 10.1002/ajmg.a.36393

Dwivedi, P. P., Grose, R. H., Films, J., Hii, C. S. T., Xian, C. J., Anderson, P. J., et al. (2013). Regulation of bone morphogenetic protein signalling and cranial osteogenesis by Gpc1 and Gpc3. Bone 53, 203–215. doi: 10.1016/j.bone.2011.06.033

Gerber, H. P., Va, T. H., Ryan, A. M., Kowalski, J., Werb, Z., and Ferrara, N. (1997). VEGF cooperates hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat. Med. 3, 623–628. doi: 10.1038/4967

Grotewold, L., and Rüther, U. (2002). The Wnt antagonist Dickkopf-1 is regulated by Bmp signaling and c-Jun and modulates programmed cell death. EMBO J. 21, 966–975. doi: 10.1093/emboj/21.5.966

Grünberg, J. R., Elvin, J., Paul, A., Hedjazifar, S., Hammarstedt, A., and Smith, U. (2018). CCN5/WISP2 and metabolic diseases. J. Cell Commun. Signal. 12, 309–318. doi: 10.1186/s12107-017-0437-z

Herter-Aeberli, I., Graf, C., Vollenweider, A., Haberling, I., Srikantan, P., Hersberger, M., et al. (2019). Validation of a food frequency questionnaire by Bmp signaling and c-Jun and modulates programmed cell death. OMICS Profiling of MBTPS2-Osteogenesis Imperfecta. Available online at: http://www.sacs.ucsf.edu/TOPO2/ (accessed May 13, 2020)

Izumi, S., Saito, A., Kanemoto, S., Kawasaki, N., Asada, R., Iwamoto, H., et al. (2012). The endoplasmic reticulum stress transducer BEEH7 suppresses apoptosis by activating the ATF5-MCL1 pathway in growth plate cartilage. J. Biol. Chem. 287, 36190–36200. doi: 10.1074/jbc.M112.373746

Jang, W. G., Kim, E. J., Kim, D. K., Ryoo, H. M., Lee, K. B., Kim, S. H., et al. (2012). BMP2 protein regulates osteocalcin expression via Runx2-mediated Atf6 gene transcription. J. Biol. Chem. 287, 905–915. doi: 10.1074/jbc.M112.215301

Johns, S. J. (2020). TOPO2, Transmembrane Protein Display Software. Available online at: http://www.sacs.ucsf.edu/TOPO2/ (accessed May 13, 2020)

Keene, D. R., San Antonio, J. D., Mayne, R., McQuillan, D. J., Sarris, G., Santoro, S. A., et al. (2000). Decorin binds near the C terminus of type I collagen. J. Biol. Chem. 275, 21801–21804. doi: 10.1074/jbc.C000278200

Kelley, R. I. (1995). Diagnosis of Smith-Lemli-Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. Clin. Chim. Acta 236, 45–48. doi: 10.1016/0009-8981(95)00638-4

Kondo, Y., Fu, J., Wang, H., Hoover, C., McDaniell, M. J., Steet, R., et al. (2018). Site-1 protease deficiency causes human skeletal dysplasia due to defective inter-organelle protein trafficking. JCI Insight 236, 45–695. doi: 10.1172/jci.insight.3121596. Available online at: http://www.sacs.ucsf.edu/TOPO2/ (accessed May 13, 2020)

Kumar, P., Paty, X., Collin, E., Rochev, Y., Rodriguez, B. J., et al. (2015). Macromolecularly crowded in vitro microenvironments accelerate the production of extracellular matrix-rich supramolecular assemblies. Sci. Rep. 5, 8729. doi: 10.1038/srep08729

Kumar, S., Hand, A. T., Connor, J. R., Dodd, R. A., Ryan, P. J., Trill, J. J., et al. (1999). Identification and cloning of a connective tissue growth factor-like CDNA from human osteoblasts encoding a novel regulator of osteoblast functions. J. Biol. Chem. 274, 17123–17131. doi: 10.1074/jbc.274.21.17123

Kumbel, S., and Savarirayan, R. (2020). “Chondrodysplasia punctata 2, X-Linked,” in GeneReviews, eds M. Adam, H. Ardinger, and R. Pagon (Seattle (WA): University of Washington).

Kuo, D. S., Labelle-Dumas, C., and Gould, D. B. (2012). Col4a1 and col4a2 mutations and disease: insights into pathogenic mechanisms and potential therapeutic targets. Hum. Mol. Genet. 21(R1), R97–R110. doi: 10.1093/hmg/ddr346

Lappe, J., Kunz, I., Bendik, I., Prudence, K., Weber, P., Recker, R., et al. (2013). Effect of a combination of genistein, polyunsaturated fatty acids and vitamins D3 and K1 on bone mineral density in postmenopausal women: a randomized, placebo-controlled, double-blind pilot study. Eur. J. Nutr. 52, 203–215. doi: 10.1007/s00394-012-0304-x

Lim, et al. (2015). Omics Profiling of MBTPS2-Osteogenesis Imperfecta. Available online at: http://www.sacs.ucsf.edu/TOPO2/ (accessed May 13, 2020)

Lindert, U., Cabral, W. A., Ausavarat, S., Tongkobpetch, S., Ladin, K., Barnes, A. M., et al. (2016). MBTPS2 mutations cause defective regulated
intramembrane proteolysis in X-linked osteogenesis imperfecta. Nat. Commun. 7:11920. doi: 10.1038/ncomms11920

Liu, C. J. (2009). The role of ADAMTS-7 and ADAMTS-12 in the pathogenesis of arthritis. Nat. Clin. Pract. Rheumatol. 5, 38–45. doi: 10.1038/nccrheum0961

Luan, Y., Kong, L., Howell, D. R., Ilalov, K., Fujardo, M., Bai, X. H., et al. (2008). Inhibition of ADAMTS-7 and ADAMTS-12 degradation of cartilage oligomeric matrix protein by alpha-2-macroglobulin. Osteoarthr. Cartil. 16, 1413–1420. doi: 10.1016/j.joca.2008.03.017

Marini, J. C., Forlino, A., Bächinger, H. P., Bishop, N. J., Byers, P. H., De Paeppe, A., et al. (2017). Osteogenesis imperfecta. Nat. Rev. Dis. Prim. 3:17052. doi: 10.1038/nrdp.2017.52

Mitsch, M. A., McDonald, J. G., Hobbs, H. H., and Cohen, J. C. (2015). Flux through a specific pathways. Mol. Cell. Biol. 35, 1038–1049. doi: 10.1128/MCB.00041-15

Mukhopadhyay, M., Shtrom, S., Rodriguez-Esteban, C., Chen, L., Tsukui, T., et al. (2017). Aspirin metabolite sodium salicylate selectively inhibits transcriptional activity of ATF6 in osteoblasts. J. Biol. Chem. 292, 11867–11876. doi: 10.1074/jbc.M116.756095

Niida, A., Hiroko, T., Kasai, M., Furukawa, Y., Nakamura, Y., Suzuki, Y., et al. (2013). Osteogenesis imperfecta. Curr. Osteoporos. Rep. 11, 203–212. doi: 10.1007/s11914-013-0149-0

Oreffner, F., Fischer, G., Happle, R., König, A., Betz, R. C., Bornholdt, D., et al. (2009). IFAP syndrome is involved in bone formation. Cell Rep. 3, 1187–1192. doi: 10.1016/j.celrep.2013.03.024

Saleh, N. K., and Saleh, H. A. (2011). Olive Oil effectively mitigates ovariectomy-induced bone loss in rats. Osteoarthr. Cartil. 19, 1090–1100. doi: 10.1016/j.jsa.2011.08.008

Saleh, N. K., and Saleh, H. A. (2013). Stress-independent activation of XBP1s and/or ATF6 reveals three new functions in severe human chondrodysplasia with progressive spinal involvement. Proc. Natl. Acad. Sci. U.S.A. 110, 14024–14029. doi: 10.1073/pnas.1305162110

Shapiro, J. R., Thompson, C. B., Wu, Y., Nunes, M., and Gillen, C. (2010). Protective effect of long-chain n-3 polyunsaturated fatty acids on bone loss due to ovariectomy in rats. Proc. Nutr. Soc. 70, 901–905. doi: 10.1017/S0029655410001417

Sharma, S. K., Rask, K. S., and Sanyal, A. K. (2013). Protective effect of long chain n-3 polyunsaturated fatty acids on bone loss in gonad-intact middle-aged male rats. Br. J. Nutr. 95, 462–468. doi: 10.1017/S0007114506003096

Shepherd, M. D., Ryno, L. M., Geneux, J. C., Moresco, J. J., Tu, L. Y., Wu, C., et al. (2013). Stress-independent activation of XBPs and/or ATF6 reveals three functionally diverse ER Proteostasis environments. Cell Rep. 3, 1279–1292. doi: 10.1016/j.celrep.2013.03.024

Tintut, Y., and Demer, L. L. (2014). Effects of bioactive lipids and lipoproteins on bone. Trends Endocrinol. Metab. 25, 53–59. doi: 10.1016/j.tem.2013.10.001
Trichopoloulou, A., Georgiou, E., Bassiaos, Y., Lipworth, L., Lagiou, P., Proukakis, C., et al. (1997). Energy intake and monounsaturated fat in relation to bone mineral density among women and men in Greece. *Prev. Med. (Baltim).* 26, 395–400. doi: 10.1006/pmed.1997.0160

Vellanki, R. N., Zhang, L., Guney, M. A., Rocheleau, J. V., Bannon, M., and Voilhuk, A. (2010). OASIS/CREB3L1 induces expression of genes involved in extracellular matrix production but not classical endoplasmic reticulum stress response genes in pancreatic β-cells. *Endocrinology* 151, 4146–4157. doi: 10.1210/en.2010-0137

Villarreal, M. M., Kim, S. K., Barron, L., Kodali, R., Baardunes, J., Hinck, C. S., et al. (2016). Binding properties of the transforming growth factor-β Coreceptor Betaglycan: proposed mechanism for potentiation of receptor complex assembly and signaling. *Biochemistry* 55, 6880–6896. doi: 10.1021/acs.biochem.6b00566

Waddington, R. J., Roberts, H. C., Sugars, R. V., Schönerr, E., Plenk, H., Daculsi, G., et al. (2003). Differential roles for small leucine-rich proteoglycans in bone formation. *Eur. Cells Mater.* 6, 12–21. doi: 10.22203/eCM.v06a02

Wang, H., Humbatova, A., Liu, Y., Qin, W., Lee, M., Cesarato, N., et al. (2020). Mutations in SREBF1, encoding sterol regulatory element binding transcription factor 1, Cause Autosomal-Dominant IFAP syndrome. *Am. J. Hum. Genet.* 107, 34–45. doi: 10.1016/j.ajhg.2020.05.006

Wang, J., Vasaikar, S., Shi, Z., Greer, M., and Zhang, B. (2017). WebGestalt 2017: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. *Nucleic Acids Res.* 45, W130–W137. doi: 10.1093/nar/gkx356

Wang, S., Qin, Y., and Bai, B. (2019). The expression, regulation, and biomarker potential of Glypican-1 in cancer. *Front. Oncol.* 9:614. doi: 10.3389/fonc.2019.00614

Wauquier, F., Léotoing, L., Philippe, C., Spilmont, M., Coxam, V., and Wittrant, Y. (2015). Pros and cons of fatty acids in bone biology. *Prog. Lipid Res.* 58, 121–145. doi: 10.1016/j.plipres.2015.03.001

Ye, J., Rawson, R. B., Komuro, R., Chen, X., Davé, U. P., Prywes, R., et al. (2000). ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* 6, 1355–1364. doi: 10.1010/S1097-2765(00)00133-7

Zhang, B., Kirov, S., and Snoddy, J. (2005). WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res.* 33, W741–W748. doi: 10.1093/nar/gki475

Zhang, C., Chen, X., Zhu, R. M., Zhang, Y., Yu, T., Wang, H., et al. (2012a). Endoplasmic reticulum stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice. *Toxicol. Lett.* 212, 229–240. doi: 10.1016/j.toxlet.2012.06.002

Zhang, G., Ezura, Y., Chervoneva, I., Robinson, P. S., Beason, D. P., Carine, E. T., et al. (2006). Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. *J. Cell. Biochem.* 98, 1436–1449. doi: 10.1002/jcb.20776

Zhang, Q., Huang, M., Wang, X., Xu, X., Ni, M., and Wang, Y. (2012b). Negative effects of ADAMTS-7 and ADAMTS-12 on endplate cartilage differentiation. *J. Orthop. Res.* 30, 1238–1243. doi: 10.1002/jor.22069

Zhang, Z., Coomans, C., and David, G. (2001). Membrane heparan sulfate proteoglycan-supported FGF2-FGFR1 signaling: evidence in support of the “cooperative end structures” model. *J. Biol. Chem.* 276, 41921–41929. doi: 10.1074/jbc.M106608200

Zhytnik, L., Maasalu, K., Reimann, E., Märtson, A., and Kõks, S. (2020). RNA sequencing analysis reveals increased expression of interferon signaling genes and dysregulation of bone metabolism affecting pathways in the whole blood of patients with osteogenesis imperfecta. *BMC Med. Genomics* 13:177. doi: 10.1186/s12920-020-00825-7

Copyright © 2021 Lim, Marfurt, Lindert, Opitz, Ndarugendamwo, Srikanthan, Poms, Hersberger, Langhans, Haus, Rohrbach and Giunta. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.