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Background/Introduction: Site-2 protease (S2P), encoded by *MBTPS2*, is a Golgi transmembrane proprotein convertase of membrane-bound transcription factors, involved in cholesterol metabolism. We previously identified an X-R form of osteogenesis imperfecta (type XVIII OI) with mutations in S2P causing impaired regulated intramembrane proteolysis (RIP) of SREBP, ATF6 and OASIS, and decreased type I collagen secretion.

Purpose: We identified probands 3 and 4 with type XVIII OI: 2y4m boy with S2Pp.N459S (c.1376A>G), and 1y5m boy with novel S2P p.L455Q (c.1364T>A) mutation.

Methods: Bone and primary osteoblasts (OB) with p.N459S were investigated with qBEI, histomorphometry, qPCR, RNAseq.

Results: Male with S2P p.N459S had LE bowing on 20 wk US. He has short stature, blue sclerae, fractures of ribs, clavicles, limbs, vertebral compressions, rhizomelia of UE and LE. His L2-L4 BMD z-score < -2 (0.167 g/cm^2).

The S2P p.L455Q mutation is associated with short stature, blue sclerae, limb fractures and deformity, undertubulated long bones with LE rhizomelia, vertebral compressions, and L1-L4 DXA z-score= -7.36 (0.128 g/cm2). Total (1574 IU/L) and bone-specific (420 mcg/L) ALP and osteocalcin (68.9 ng/ml) were elevated. Oasis processing in proband FB revealed decreased 50kD S1P/S2P cleavage product.

Cortical bone from S2Pp.N459S proband had notable marrow fibrosis and was not hypermineralized, distinct from classical OI. Histomorphometry revealed increased osteoblast (14.1%; control 8.5±4.1) and osteoid surface (46.8%; control 34±6.7). *In vitro,* the S2P p.N459S mutation hampered osteoblastogenesis. Early osteoblast markers were downregulated in primary OB, whereas, late osteoblast/early osteocyte markers were upregulated. *In vitro* mineralization was severely delayed in proband OB. Transcript profiling revealed that p.N459S alters expression of genes encoding ECM constituents and involved in ECM organization.

Conclusion(s): These *MBTPS2* missense mutations support a critical role of RIP in normal bone development. The distinctive features of type XVIII OI bone tissue and OB will reveal insights into the tissue-specific mechanism of RIP.

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The skeletal effect of post-natal treatment with N-acetylcysteine in a diastrophic dysplasia mouse model

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Background/Introduction: Diastrophic dysplasia is a recessive skeletal dysplasia caused by mutations in the *SLC26A2* gene encoding for a sulfate/chloride antiporter of cell membrane. Functional impairment of the transporter causes reduced sulfate uptake leading to cartilage proteoglycan (PG) undersulfation. Even if intracellular sulfate is mainly dependent on extracellular uptake, a small amount comes from the catabolism of sulfur-containing amino acids and other thiols.

Purpose: Here we are investigating a treatment with N-acetylcysteine (NAC), a cysteine derivative, in dtd mice.

Methods: Mice were treated twice a day with hypodermic injections of 250 mg NAC/Kg body weight for 21 days from birth. The effect of NAC was evaluated by cartilage PG sulfation analysis, X-rays morphometry, histology of the tibia growth plate and DEXA.

Results: At the end of the treatment, cartilage PG sulfation was significantly increased in treated dtd mice compared with the placebo dtd group (84.50% vs 80.40% sulfated disaccharides, respectively; $P < 0.05$). The length of different skeletal elements (tibia, femur, radius, vertebrae and hip) was increased showing a skeletal improvement in treated dtd mice. This improvement was also confirmed by a significant increase of body weight (6.22 g vs 5.08 g; $P < 0.01$) and length (49.17 mm vs 44.23 mm; $P < 0.001$) in dtd treated mice compared to dtd untreated ones. Histology of the growth plate showed an amelioration of its architecture in NAC treated dtd mice compared with untreated animals, further suggesting correction of the endochondral ossification process. The improvement of the bone phenotype of NAC treated dtd mice compared with untreated ones was demonstrated by an increase of femur and tibia BMC (3.56 vs 1.25 mg; $P < 0.001$).

Conclusion(s): Overall, our results demonstrated that NAC is an alternative source of intracellular sulfate through its catabolism paving the way for a pharmacological treatment of diastrophic dysplasia patients.

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Changes in ECM components in Osteogenesis Imperfecta type V

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Background/Introduction: Osteogenesis Imperfecta type V (OI-V) is rare genetic bone disease caused by an autosomal dominant mutation at the 5'UTR of IFITM5 resulting in an addition of 5 amino acids at the N-terminus of the gene product. Clinically, OI-V patients present various degrees of bone fragility and deformity, and with or without characteristic heterotrophic bone growth and hyperplastic callus formation. However, in line with current reports, our clinical specimens near fracture sites show a consistent presence of mesh-like bone lamella, especially around Haversian canals, and increased lacuna density, suggesting problems in extracellular matrix (ECM) organization and/or osteoblast and osteocyte functions.

Purpose: Since mesh-like lamella is consistent and, based on other reports, specific to type V OI, to understand more on the disease mechanism, we decided to investigate the differences in ECM and cellular proteins produced by osteoblast-like cells cultured from three OI-V patients' and three non-OI individuals' bone samples, and validated some of the results with immuno-staining of clinical specimens.

Methods: Osteoblast-like cells were cultured in osteogenic condition, and samples were collected at regular time points for Alizarin Red staining and protein isolation followed by mass spectrometry. Newly synthesized proteins were labeled with heavy isotopes for 24 hours using SILAC system.

Results: OI-V cells show a significantly lower H/L ratio in majority of detected proteins compared with non-OI cells, including most of the