The Wnt1G177C mutation impairs mechanically induced bone formation through nuclear receptor 4a2

Mubashir Ahmad1, Melanie Haffner-Luntzer1, Astrid Schoppa1, Timur A. Yorgan2, Michael Amling3, Thorsten Schinke2, Anita Ignatius4

1Institute of Orthopedic Research and Biomechanics, University Medical Center Ulm, 89081 Ulm, Germany
2Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

Email of Presenting Author: ahmad.mubashir@uni-ulm.de

Disclosures: MHL: ORS ISFR section office

INTRODUCTION: Mechanical loading triggers bone formation. While the precise mechanism driving this phenomenon remains elusive, accumulating evidence suggests the indispensable role of the Wnt signaling pathway in facilitating the anabolic response. In this study, our objective was to investigate the effects of the Wnt1G177C mutation on mechanically induced bone formation, a mutation which leads to osteogenesis imperfecta type XV in humans. To this end, we combined in vivo and in vitro experiments using homozygous Wnt1G177C mice and osteoblast cultures, respectively, to unravel the signaling cascades through which the Wnt1G177C mutation influences bone formation induced by mechanical stimuli.

METHODS: The experimental procedures for in vivo mechanical loading of the mouse ulna were conducted with approval from the animal care committee of Baden-Württemberg (Regierungspräsidium Tübingen, No.1566). For ex vivo calibration of the loading protocol, strain gauges were affixed to the lateral surface of the ulna in 12-week-old female mice of both the Wnt1+/+ and homozygous Wnt1G177C groups (n=6). The right ulnae were positioned in the loading apparatus to measure the load needed for a peak surface strain of 2000 μstrain. Cyclic axial compression of the right ulnae was conducted, applying a frequency of 2 Hz and a maximum load of 2.0 N and 0.5 N in 12 consecutive days, each for one minute. The left ulnae were utilized as non-loaded controls. For micro-computed tomography (μCT) and RNAseq analysis, the mice were euthanized on day 16 and day 4, respectively. The ulnae were subjected to μCT using Skyscan 1172 with an 8 μm resolution. A 1 mm thick section of the medio-diaphyseal cortical bone, starting 3 mm distally from the ulnae head, was designated as the region of interest. Regarding RNAseq analysis, the ulnae were cleared of the surrounding tissue and centrifuged (12500 rpm; 1 min) to remove bone marrow. The subsequent steps included RNA isolation using the RNAeasy kit, evaluation of RNA concentration and integrity through 2100 Bioanalyzer and RNA 6000 Nano kit. RNA sequencing using Illumina technology was carried out by Novogene. Gene expression quantification was achieved by read count method. Principle component analysis (PCA) was implemented to evaluate sample variability and repeatability using RStudio with pcaExplorer. The overall distribution of differentially expressed genes (DEGs) was illustrated using volcano plots. Gene ontology (GO) analysis of DEGs was performed using Metascape. Furthermore, mechanical stimulation experiments were conducted on primary murine osteoblasts derived from the long bones of 12-week-old female Wnt1+/+ and Wnt1G177C mice (n=5). The cells were seeded onto Flexcell glass slides at a density of 20,000 cells/cm². Cells were either left untreated (static control) or mechanically stimulated by laminar fluid flow (LFF) applying a shear stress of 10 dynes/cm² for 1h using the Flexcell streamer system. RNA samples were collected immediately (0h) after LFF. The RNA isolation from primary osteoblasts involved extraction using RNAeasy kit, CDNA synthesis using 1 μg RNA, followed by qPCR on the QuantStudio 5 system. The relative mRNA concentrations, normalized to beta-2 microglobulin, were calculated using the ΔACT method.

RESULTS: To elucidate the functional implications of the Wnt1 mutation (Wnt1G177C) in the bone’s anabolic response to loading, we exposed the ulnae of 12-week-old female mice from both Wnt1+/+ and Wnt1G177C groups to mechanical loading (Fig. 1A). μCT analysis revealed that ulnae loading substantially increased cortical thickness in Wnt1+/+ control mice, whereas this effect was attenuated in Wnt1G177C mutant mice (Fig. 1B-C). RNAseq analysis revealed 1704 DEGs influenced by the Wnt1G177C mutation during mechanical loading (Fig. 1D). By investigating these DEGs with Metascape analysis, we identified various pathways, involved in skeletal system development, extracellular matrix organization, blood vessel development, regulation of neuron projection development, and mesenchyme development, that were modulated during loading. To discern which of these DEGs were specifically governed by Wnt1G177C mutation, we compared them with datasets from RNAseq performed on primary osteoblasts, where Wnt1 was deleted using siRNA, followed by LFF-induced stimulation. This analysis highlighted 16 common genes, with one consistently demonstrating dependence on both Wnt1G177C mutation and stimulation, was nuclear receptor subfamily 4, group A, member 2 (Nr4a2) (Fig. 1E). Depletion of NR4A2 in human mesenchymal stem cells (MSCs) confirmed its positive role in promoting osteoblast differentiation. These findings demonstrate that Wnt1G177C mutation impairs mechanical loading-induced bone formation through modulation of Nr4a2 expression.

DISCUSSION: Our research delved into the clinically relevant Wnt1G177C mutation on bone response to mechanical loading in mice. Ulnae loading triggered a substantial increase in cortical thickness in Wnt1+/+ control mice, whereas this response was compromised in mice harboring the Wnt1G177C mutation. Analysis using RNAseq revealed 1704 DEGs influenced by the Wnt1G177C mutation during mechanical loading. Further investigation pinpointed Nr4a2 as a key gene affected by both the mutation and loading, with depletion of NR4A2 confirmed it promoting role on osteoblast differentiation. This study underscores the role of Wnt1G177C mutation in disrupting bone formation prompted by mechanical loading through modulation of Nr4a2 expression.

SIGNIFICANCE: Our study highlights the significant impact of the clinically relevant Wnt1G177C mutation on impairing the bone’s ability to respond to mechanical loading, leading to decreased cortical thickness. Through the identification of molecular pathways and the key involvement of Nr4a2, this research provides valuable insights into the complex mechanisms governing bone formation in response to mechanical stimuli. These findings offer potential avenues for therapeutic interventions targeting these pathways.