Identification of a novel mutation in heritable osteonecrosis of the femoral head

Tracy Wang¹³, Wayne Mah¹, Edward Harvey¹², Jacek Majewski³, David Rosenblatt³, Chantal Seguin¹⁴

¹Bone Engineering Labs, Surgical Research, Research Institute McGill University Health Center, Montreal, QC, Canada, ²Department Surgery, Division Orthopaedic Surgery, McGill University Health Center, Montreal, QC, Canada, ³Department of Human Genetics, McGill University, Montreal, QC, Canada, ⁴Department of Medicine, Divisions Haematology and Oncology, McGill University Health Center, Montreal, QC, Canada

Background: Osteonecrosis of the femoral head (ONFH) is a debilitating disease that arises from an interruption of blood flow to the femoral head, causing the bone to die and eventually collapse [1]. The majority of cases of ON are idiopathic, but there have been several families of Asian descent identified with autosomal dominant inherited forms of the disease. Heritable forms of ONFH has been associated with a c.3508G>A (p.G1170S) mutation in exon 50 of the COL2A1 gene [2]. A Canadian family of Greek origin has been identified with familial ONFH in which four of six siblings have advanced bilateral ONFH, which suggests that the disease is dominant.

Aim: The study aims to identify the causal mutation of ONFH in this family.

Material and methods: An examination of X-ray radiographs of the proband and three siblings confirmed that all affected individuals have advanced bilateral ONFH. Extensive skeletal surveys do not reveal other bone abnormalities. DNA from four affected and one unaffected family members was extracted from blood or saliva samples. Genetic analyses included screening for the common COL2A1 mutation, whole exome sequencing of the proband, and segregation analyses on affected and unaffected individuals. A cohort of 54 patients with sporadic ONFH were used for DNA sequence comparison. Protein expression was assayed using immunoblots and densitometry probing.

Results: The proband was wild type for the common COL2A1 mutation. Exome sequencing followed by segregation analyses indicated that affected individuals have a heterozygous c.2570_2573delCCGC (p.R828WfsX3) frameshift deletion in the last exon of TRPV4 that was absent in the unaffected individual. This mutation was absent in all 54 sporadic samples. Preliminary immunoblots and densitometry probing wild-type TRPV4 expression in patient and control fibroblasts found a 70% decrease in the patient. As the mutation is present in the last exon, the mutant allele likely escape degradation by nonsense-mediated decay. This mutation is novel and has never been reported in association with osteonecrosis.

Figure 1: Protein expression in control vs. patient fibroblasts (A) TRPV4 (B) loading control C) relative TRPV4 expression. D) c.2570_2573delCCGC frameshift deletion in proband

Conclusions and clinical implication:

This is the first reported case of heritable ONFH in patients not of Asian descent and the first report of a novel mutation in TRPV4 gene in association with osteonecrosis. Its role in Ca²⁺ influx and intracellular Ca²⁺ signaling may contribute to osteonecrosis-associated functions such as osteoclast and vascular regulation which merit further study. Our findings expand the scope of the existing knowledge of heritable causes of ONFH.

References: