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Impact of Trp2 allele mutation of α2 chain in collagen IX on the structural integrity of human annulus fibrosus

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INTRODUCTION: A preliminary study has suggested that Trp2 allele mutation in α2 chain of collagen IX (*COL9A2*) may attribute to early degenerative disc desease[2]. In intervertebral discs (IVD), collagen IX is found in close association with collagen II and it is hypothesized that collagen IX may play an important role in maintaining the structural integrity of the discs through its covalent cross-links with collagen II[2]. Collagen II is present in all three components of the IVD i.e. annulus fibrosus (AF), nucleus pulposus and end plates. This abstract focuses only on the AF. In the outer edge of the AF, the concentration of collagen II is very low[3]. The concentration of collagen II is known to increase from the outer edge towards the interior of AF in radial direction. Thus we have arrived at a hypothesis that, testing the mechanical properties of the inner AF region may enable us to find the influence of Trp2 on the mechanical properties of AF. Studies have been carried out in the past to determine the overall mechanical properties of the AF[1], but there is not much work done to find the microscopic mechanical properties of inner AF collagen II. Thus a study in the microscopic level is essential to figure out the fine differences in mechanical properties that are expected to be caused by mutations such as the Trp2 allele.

PROPOSED METHODS: We aim to test and compare 20 samples each from the Trp2 mutated non-degenerated and normal non-degenerated controls of human inner AF specimens. The samples will be collected from patients who undergo scoliosis correction procedure. The incidence of Trp2 among the south Chinese population is about 20%, which makes it easily possible for collecting both mutated and normal discs.

The annulus part of the disc will be carefully removed and genotyped before storing at -80° C. Thin sections of inner AF will be microtomed to a thickness of approximately 0.5mm using a cryostat. Individual fibers of length approximately 1.5mm will be dissected from the slice under a binocular microscope. The experimental setup and the tensile testing protocols from a previous study on rat muscle fibers will be adopted with some modifications[4]. The fibers will be held between the force and displacement transducers by gluing the ends. The movements will be controlled with a computer and servo controller. Based on our pilot study conducted on bovine AF specimens, a 2.5 N force transducer of frequency resonance 1 kHz was found to be appropriate for this purpose and the same will be used in the actual study. The force and displacement will be visualized in an oscilloscope and recorded into a computer for further analysis.

POSSIBLE OUTCOMES AND DISCUSSION: The outcome of this study can be of three types. 1) Trp2 affected AF fibers are weaker than non-Trp2 affected AF fibers. 2) Trp2 affected AF fibers are stronger than non-Trp2 affected AF fibers. 3) No significant difference between the tensile mechanical properties of Trp2 affected and non-Trp2 affected AF fibers. Outcome (1) will prove that, Trp2 plays a significant role in weakening the AF and thus leading to DDD. Outcome (2) will suggest that the role played by Trp2 is benign as far as the tensile mechanical properties of AF are concerned. Outcome (3) may mean one of the following two (a) Trp2 does not affect the mechanical properties of AF; (b) the testing method is not sensitive enough to discriminate the role played by Trp2. Outcome (3) will thus suggest for a nano-level testing involving individual collagen II fibrils.

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