Ectopic bone formation in the endplate of apoE deficient mice.

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INTRODUCTION:
Degenerative disc disease is a leading cause of lower back pain in adults, and can be especially problematic for obesity. Were it because that extra weight puts extra strain on the spine or whether obesity or lipid metabolism is related to disc degeneration is unknown. Apolipoprotein E (ApoE) is a class of apolipoprotein found in the intermediate-density lipoprotein and chylomicron that transports lipoproteins, fat-soluble vitamins, and cholesterol into the lymph system and then into the blood. ApoE was initially recognized for its importance in lipoprotein metabolism and cardiovascular disease. More recently, it has been studied for its role in several biological processes not directly related to lipoprotein transport, including Alzheimer's disease, immunoregulation, and cognition. Besides, increased bone mass was found in apoE-deficient mice. ApoE+/- mice display an increased trabecular bone volume in the vertebra and tibia at 8 months of age, showing that ApoE has a physiologic function in bone remodeling. Increasing evidences showed that bone mineral density of the vertebral body is related to intervertebral disc (IVD) degeneration. To investigate whether apoE is expressed in the intervertebral disc and whether apoE is involved in disc degeneration, lumbar spines were isolated from age matched apoE knock-out mice and apoE wildtype mice to compare the histology, gene expression, and immunohistochemical properties.

METHODS:
Animal: ApoE knock-out (KO) and apoE wildtype (WT) mice were purchased from Jackson laboratories. Euthanasia was done by cervical dislocation after anesthesia with sodium pentobarbital (3-4 mg/100 g IP). All procedures of this study were previously approved by the Institutional Animal Care and Use Committee at the University of Virginia.

Histological analysis: Lumbar Spine samples were fixed with 4% paraformaldehyde for 2 days followed by 0.25M EDTA decalcification for 4 weeks. The decalcified samples were dehydrated by treatment with a series of graded alcohols and embedded in paraffin. Samples were then cut into 6µm sections, rehydrated, and stained with safranin-O and fast green for detection of proteoglycan.

Immunohistochemistry: Briefly, after deparaffin and antigen retrieval, serial sections were incubated with antibodies specific for ApoE and Osteocalcin (OCN) (Santa Cruz) in biotin blocking solution (Vector Labs) overnight at 4°C. Binding of the primary antibodies was detected by Vectastain Kit (Vector Labs). Negative controls, which consisted of omission of the primary antisera, were uniformly negative. Positive controls were used to confirm immunostaining.

Real-time PCR: Total RNA was isolated from disc tissues and cells with the RNeasy Kit (Qiagen). First-strand cDNA synthesis was performed using 0.5µg total RNA and the iScript cDNA Synthesis Kit (Bio-Rad). For real-time PCR, the iQ SYBR Green Supermix Kit (Bio-Rad) was used. The gene expression of 18S was used to normalize expression.

RESULTS:
Ectopic bone formation was found in the endplate of apoE knock-out mice at age of 10 wks and 15 wks as shown in Fig 1. While, no bone formation was found in the age-matched wildtype counterparts. No significant differences were found between the apoE knock-out and wild-type mice at age of 5wks. OCN immunostaining confirmed the ectopic bone formation in the endplate of apoE KO mice at age of 10 wks and 15 wks (Fig 2). In Fig 3, positive immunostaining for apoE was found in the endplate of wild-type C57BL/6 mice. No positive staining was found in the annulus fibrosus.

In accordance with OCN immunostaining, the expression of bone formation markers, such as Runx-2 and OCN, was up-regulated to 1.84-fold and 1.54-fold, respectively (Fig 4A); meanwhile, matrix degrading enzymes, such as MMP-3, MMP-9 and MMP-13, were also increased to 1.73-fold, 2.26-fold and 1.47-fold, respectively, compared with apoE wild-type mice IVD cells (Fig 4B).

REFERENCES: