Polymorphic variants in ALDH1A2 determine the expression level of ALDH1A2 and CYP19A1 in the cartilage of patients undergoing trapeziectomy for severe thumb osteoarthritis

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Background: A recent Genome Wide Association Study (GWAS) in hand OA has uncovered the association of two SNPs markers rs4238326[C] and rs3204689[C] with severe hand osteoarthritis. The two variants are all located within a single linkage disequilibrium block that contains one ALDH1A2 (aldehyde dehydrogenase 1 family member A2). This gene encodes the enzyme that irreversibly catalyzes the production of all-trans retinoic acid (atRA), and which regulates numerous physiological processes involved in embryonic limb development and in maintenance of adult tissues.

Trapeziectomy is a recognised surgical treatment for patients with intractable base of thumb OA. In this study, we developed a method to extract good quality RNA from the articular cartilage of the trapezium, the small bone at the base of the thumb. We investigated the correlation between the presence of variant ALDH1A2 alleles (snp3204689, and snp4238326) and mRNA levels of ALDH1A2 and a series of retinoic acid dependent genes and inflammatory response genes.

Methods: We identified 26 patients, with base of thumb OA, scheduled to undergo trapeziectomy at the Nuffield Orthopedic Centre, Oxford, UK. Following written informed consent, the removed intact trapezium was kept for research. Within one hour of collection, articular cartilage was dissected from the trapezium and snap frozen in liquid nitrogen. RNA was extracted using the RNeasy Micro Kit from QIAGEN. RNA yield and quality were tested using an Agilent Bioanalyzer and Nanodrop Spectrophotometer. The RNA integrity number (RIN) values were above 7 for all samples. Genomic DNA was extracted from the trapezium bone, and the allelic region of interest was amplified using specific primers. The purified PCR products sequenced and their genotype uncovered. The expression levels of selected genes including retinoic acid-dependent genes (e.g.CYP19A1, CYP26a, CYP26b, CYP26c, retinoic acid receptor- RARα, β, and γ) as well as inflammatory genes (including ADAMTS4, ADAMTS5, CCL2, MMP13) were tested by RT-PCR using TaqMan Low Density Arrays (TLDA) microfluidic cards.

Results: We detected a high prevalence of the two polymorphic variants (76.9%) in ALDH1A2 within the 26 patients. We classified the samples into five groups based on the number of variant alleles (see chart below). We found the mRNA level of ALDH1A2 was significantly lower in the Homozygous group compared to the wild

type (p = 0.02, T-test). Among the retinoic acid dependent genes, the only one that correlated significantly with genotype was CYP19A1. We also found trends in the regulation of several other retinoic acid dependent and inflammatory response genes, but these were not statistically significant.

Number of variant alleles (C)	0	1		2			3		4
rs4238326 T → C	WT	Heter	WT	WT	Homo	Heter	Heter	Homo	Homo
rs3204689 G → C	WT	WT	Heter	Homo	WT	Heter	Homo	Heter	Homo
Total patients (26)	6	0	2	1	0	8	0	1	8
Number of Female:Male	3:3	——	1:1	1:0	——	5:3	——	0:1	8:0

Conclusions: Our data shows that polymorphic variants in ALDH1A2 are highly prevalent in our hand OA population and are associated with significantly lower levels of ALDH1A2 in hand OA cartilage. The lower expression level of ALDH1A2 in the variants is predicted to decrease cellular atRA levels, and at least one retinoic acid-dependent gene, CYP19A1, correlated with ALDH1A2. CYP19A1 encodes the enzyme that catalyzes the last step of the estrogen and testosterone biosynthesis pathway. This may potentially point to a role for retinoic acid in the regulation of sex hormone levels either systemically or in the tissue.