# Metabolomic characterization of human prostate cancer with intact tissue MRS

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**Introduction:** Metabolomic profiles measured from intact tissue high resolution magic angle spinning (HRMAS) MRS have hypothesized improved diagnostic and prognostic potentials in differentiating tissue specimens with and without cancer glands, and patient pathological stages for biopsy proven prostate cancer (PCa) patients, when compared with individual metabolites. The current study is designed to test the hypothesis from evaluation of independent PCa patient cohorts.

**Methods:** A total of 390 human prostate tissue samples from 175 biopsy proven PCa patients have been included in this report. Among them, 199 samples from 82 patients formed a training cohort, and are tested against the testing cohort (191 samples from 93 patients). Detailed patient information is listed in Table 1. Within these 390 samples, 46 were characterized with PCa glands, while 344 samples represented histologically benign tissue samples from PCa patients. Furthermore, among these histo-benign samples, 204 samples were obtained from all three prostate zones of 68 patients from which metabolic differences of prostate zones were analyzed.

**Table 1.**

| Patient Age | Gleason Score (GS) | # of Patients | Pathological Stage (T) | # of Patients | T and GS | # of Patients |
|---|---|---|---|---|---|---|
| M=58.99429 | GS5 | 8 | T2 | 138 | T2, GS6 | 97 |
| SD=6.77173 | GS6 | 107 | T3 | 37 | T2, GS7 | 32 |
| Min=41 | GS7 | 48 | | | T3, GS6 | 10 |
| Max=75 | GS8 | 6 | | | T3, GS7 | 16 |
| | GS9 | 6 | | | T2, GS5 | 8 |
| Pn = 175 | | | | | T3, GS8,9 | 11 |
| Sn = 390 | | | | | T2, GS8 | 1 |

*MR Spectroscopy.* A Bruker (Billerica, MA) AVANCE spectrometer operating at 600MHz (14.1T) was used for all MR experiments. Tissue samples were placed into a 4mm rotor with 10µl plastic inserts. 1.0µl $D_2O$ was added for field locking. Spectra were recorded at 3ºC with the spectrometer frequency set on the water resonance, and rotor-synchronized experimental protocols with or without DANTE sequence with spinning at 600 and 700Hz (±1.0Hz). 32 transients were averaged at a repetition time of 5s. Spectra were analyzed by an in-house MatLab based program. All FIDs were subjected to 1 Hz apodization; metabolite resonances were integrated and calibrated according to the external and internal standards.

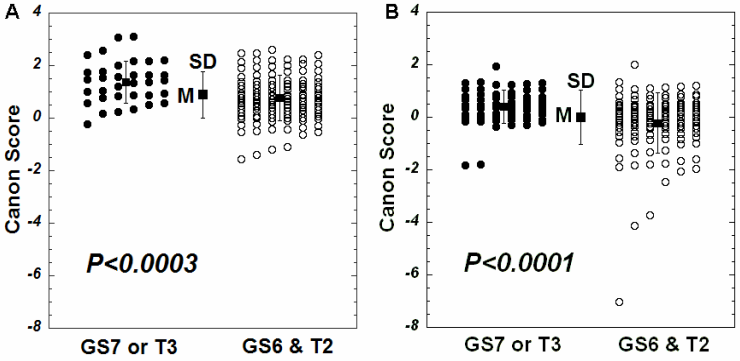


Figure 1. Prostate cancer metabolomic profiles calculated for the testing cohort (A) can differentiate PCa of T2, GS6 from GS7 or T3 according to the parameters obtained from the training cohort (B).

**Table 2.**

| Metabolite | Transitional Zone (TZ) Mean | Peripheral Zone (PZ) Mean | Central Zone (CZ) Mean | p-Value TZ vs. PZ | p-Value TZ vs. CZ |
|---|---|---|---|---|---|
| Choline | 1.8919 | 0.5107 | 0.6652 | 0.0002 | 0.0009 |
| Creatine | 1.4130 | 0.4346 | 0.3379 | 0.0005 | 0.0001 |
| Alanine | 0.5373 | 0.1453 | 0.1123 | 0.0003 | 0.0001 |
| Lactate | 10.8983 | 3.6486 | 2.5345 | 0.0006 | <0.0001 |

**Results and Conclusions:** 1) Metabolomic profiles represented by the Canonical Score obtained from Canonical Analysis of the training cohort (cancer, 0.92+/-0.22; histo-benign, -0.10+/-0.07; $p<0.0001$) can differentiate the testing cohort (cancer, 0.89+/-0.23; histo-benign, 0.25+/-0.08, $p<0.0081$). 2) The Metabolomic profile obtained from histo-benign samples of the training cohort can differentiate PCa of Gleason score (GS) 6 and pathological stage II (T2) from those of either GS 7 or T3, as shown in Figure 1. 3) After Bonferroni corrections, statistically significant differences of resonance intensities measured for three prostate zones and normalized by the volume percentage of tissue epithelia were found in 14 from the 36 regions evaluated with two-way ANOVA (Table 2).

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