Editorial Comment to: Prostate Cancer Antigen 3 Gene Expression in Peripheral Blood and Urine Sediments from Prostate Cancer and Benign Prostatic Hyperplasia Patients versus Healthy Individuals

I read with great interest the manuscript entitled "Prostate Cancer Antigen 3 Gene Expression in Peripheral Blood and Urine Sediments from Prostate Cancer and Benign Prostatic Hyperplasia Patients versus Healthy Individuals". This manuscript has some important shortcoming and drawbacks which should be clarified for readers. I summarize them, otherwise it take lots of thorough notes.

1. The most important one is study sample size. The authors have acknowledged this issue, but the study sample size is very small to draw any conclusion. The minimum requirement sample size for gene expression study is 150.[1] In present study the total number of patients with prostate cancer (PCa) is 24. Even this number of patients has been categorized into five subgroups, which made the obtained results totally questionable. Factors that affect power and sample size calculations include variability of the population, the desired detectable differences, the power to detect the differences and an acceptable error rate. Calculating of sample size and reporting the study power are mandatory in each gene expression study.
(see: http://www.cscu.cornell.edu/news/statnews/stnews41.pdf).
Usually we are interested in calculating the sample size needed to have the effect size (or odds ratio) in the range of 1.5-2.0 with at least 80 percent power under a dominance model. Quanto can compute the required sample size. The following link has an example to see how Quanto works. http://www.cscu.cornell.edu/news/statnews/stnews71Quantoexample.pdf

2. The study lacks of even scientific short literature review. There are some nationwide studies regarding the prevalence of PCa in Iranian men.[2,3] None of them has been cited in present study. Relying only for two congress abstracts[4,5] is not acceptable in scientific era. The authors claimed that, the incidence of PCa has been increased in Iran during last decade. There is no reference for this statement. Indeed according to the two nationwide population based studies[2,3] the incidence of PCa in Iran is much lower than that in Europe and America and even some Asian countries.

3. The cancer gene expression study should comply with standards. The most important one is "STrengthening the REporting of Genetic Association Studies (STREGA)". None of the criteria which have mentioned in STREGA recommendation has been addressed in present paper. The scientific background and rationale for the investigation is not clear. The issue of prostate cancer gene 3 (PCA3) expression in prostatic diseases and in normal individuals already has been studied in more than one hundred scientific papers with large sample sizes.[6-10] I don't know which new data has been added to the literature by this paper.

4. Classic statistical issues such as appropriate, study sample size, replicate structure, statistical significance and outlier determination are important issues in the planning and analysis of gene expression studies. The authors claimed that, 95% confidence interval (CI) has been calculated. But, indeed there is any 95% CI reported in this manuscript. An analysis solely based on fold change does not allow the assessment of significance of expression differences in the presenceof biological and experimental variation, which may differ from gene to gene. This is the main reason for using statistical tests to assess differential expression. Generally, one might look at various properties of the distributions of a gene's expression levels under different conditions. The authors mentioned that, relying the results of this study, may omit the necessity of prostate biopsy for detecting PCa. I think this recommendation is absolutely impractical. Up to now the expression and frequencies of more than 100 genes have been studied regarding PCa.[11-17] But none of them can be replaced by prostate biopsy. I have a question. Does a 60 years old men with serum PSA level of 8 ng/mL, and negative PCA3 gene expression need prostate biopsy or not? In addition a linear regression model should be used to adjust confounding factors such as age, body mass index, occupational status, educational level, smoking status and etc. All of them should put in a multiple regression analysis model. What is more important in gene association study is correlation between gene single nucleotide polymorphisms (SNP) and related disease. Regarding PCA3, one should calculate PCA3 scores. PCA3 score is generated from the ratio of PCA3 mRNA level to prostate specific antigen (PSA) mRNA level, through which PCA3 expression is normalized with the PSA expression used as a housekeeping gene.

Presently, the first discovery of genetic determinants based on a genome-wide study for PCA3, which has been performed by Chen et al.[18] They included 1371 men in their study. "Two SNPs, rs10993994 in β-microseminoprotein at 10q11.23 and rs10424878 in kallikrein-related peptidase 2 at 19q13.33, were associated with PCA3 score at genome-wide significance level (P = 1.22 x 10-9 and 1.06 x 10-8, respectively). Men carrying the rs10993994 "T" allele or rs10424878 "A" allele had higher PCA3 score compared with men carrying rs10993994 "C" allele or rs10424878

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"G" allele (β = 1.25 and 1.24, respectively)."

5. RT-PCR methods can provide quantitative information regarding mRNA expression levels, and individual gene identities can be detected. The major drawback is that it would be difficult for academic laboratories to automate, and there is always some uncertainty regarding the detection of each gene being investigated. High-throughput sequencing technology is rapidly becoming the standard method for measuring RNA expression levels (aka RNA-seq).[19]

The results of present study should be interpreted very cautiously. Genome-wide association studies (GWAS) are needed to identify SNPs that are associated with the urine and blood levels of PCA3.

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