Author's response to reviews

Title: the effect of tobacco, XPC, XPD and XPG genetic variants in bladder cancer development

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Author's response to reviews:

Dear Editor,

Enclosed we are sending a revised version of the article “The effect of tobacco, XPC, ERCC2 and ERCC5 genetic variants in bladder cancer development” that we have modified according to the comments of the referees:

Answering point by point to their questions:

Reviewer 1

Major compulsory revision:

1. There is no information on how authors calculated sample size for the study. Considering numbers of individuals recruited (193 patients and 193 controls) and a lower minor allele frequency of XPG (rs17655) polymorphism, it is really important to estimate whether current sample size successfully predict odds for bladder cancer incidence. The issue becomes more serious when data get stratified in sub-groups like smoking habits and sample size further reduces in the cells of a 2x2 table. Therefore, author should provide details of sample size calculation and should apply appropriate correction for small sample size, where required.

Answer to comment 1:

I think that the current sample size can successfully predict odds for bladder cancer incidence for many reasons:
- First reason: this pathology was not very frequent in Tunisian population. Indeed only 200 subjects were annually diagnosed for bladder cancer in Tunisia (this effective is equal to which analysed in our data)

- Second reason: Tunisia is a very small country with less than 11 millions peoples

- Third reason: the frequencies of studied alleles were high (more than 30%).

2. The recruitment criteria for controls is not clear, whether they were work out for absence of any urinary tract disease and confirmed by cyctoscopy or by any other means is not clear.

Answer to comment 2

To clarify how controls were recruited we added this sentence in the material and methods section: “Controls were recruited daily from patients newly diagnosed and treated at the same urology department for benign diseases, mainly prostatic hyperplasia, cystitis and urolithiasis. Patients with cancer, or liver or renal diseases, were excluded”.

3. Another major issue is correction for multiple comparisons. I think author may like to use Bonferroni’s correction when analyzing genotype and tobacco use or tumor stage. I guess, most of the marginal association will turn into non-significant association after this correction, which is a major lacuna of this study.

Answer to comment 3:

Corrected probability values (pc) were determined using Bonferroni’s correction by multiplying each p value by the number of allele or haplotype comparisons made. We have done this correction for table 1 and the corrected p value is 0.04. For the regression analysis we think that the Bonferroni’s correction was not essential because all of obtained p value were very significant.

4. There are reports which suggest that patients with same stage of tumor but with different grade, behave differently to treatment and tumor invasion (Ref. Scand J Urol Nephrol. 2003;37(3):195-201). Therefore, following patient classification pattern, presented in that paper, according to stage and grade both, may provide some fruitful information.

Answer to comment 4:

I agree with you. Indeed among superficial bladder cancer, the high-grade tumors progress to invasive disease and represent a high risk for death from disease. The management of bladder cancer is dependent on tumor stage and grade. The pTa and pT1 tumors are removed by transurethral resection with or without Bacillus Calmette Guérin (BCG) therapy, whereas invasive tumors are treated by radical cystectomy with or without postoperative chemotherapy. For this we have added the classification of tumors according to both stage and grade in the material and methods section: “13 carcinoma in Situ (CIS), 34 pTa GI, 12 pTa GII, 3 pTa GIII, 53 pT1 GII, 34 pT1 GIII and 44 invasive tumors (# pT2)”. 
5. Though they adjusted OR for age and gender, they have completely ignored presence or absence of smoking.

Answer to comment 5:
In the multinomial logistic regression they are 3 types of variables:
- depend (controls/cases; low stage/high stage; low grade/ high grade)
- factors (risk factors )
- covariate (sex, age)

The tobacco status can be analysed as “factors” or as “a covariate factors”. However many reported data have reported that tobacco increases the risk for bladder cancer, for this we used tobacco status as a “risk factor” but not as “ a covariate factor” and we adjusted OR only for age and gender.

6. In most of the complex diseases such as bladder cancer, many genes are involved and they interact with each other to alter disease phenotype. Therefore, analyzing combined effect of these polymorphisms would be interesting and useful.

Answer to comment 6:
I agree with you that analyzing combined effect of many polymorphisms would be interesting and useful. Indeed we have reported in other papers that genetics polymorphisms affecting folate metabolising pathway or the xenobiotic metabolising enzymes act additively to increase the risk of bladder cancer (Ouerhani et al, 2009 and Rouissi et al 2009). In this work we have analysed the combined effect of XPC, XPG and XPD polymorphisms in bladder cancer development and we haven’t report any additive effect (data not shown). To more elucidate this concept we prepare a review which try to study the effect of all previously analysed polymorphisms in association with bladder cancer for the Tunisian population.

Minor Essential Revisions
1. Page 20, par 2, line 7- “This results confirms…..” should be “These results confirm” .

Answer to comment 1:
The sentence is corrected

2. “Smokers patients” should be replaced with smoker patients at all places.

Answer to comment 2:
“Smokers patient” was replaced by “Smoker patients” in all places.

3. There are many sentences need to be rephrased.

Answer to comment 3:
The incorrect sentences were rephrased

4. I would suggest following HUGO nomenclature through out the manuscript.

Answer to comment 3:
We have used the HUGO nomenclature in all the text:
- XPD was replaced by ERCC2
- XPG was replaced by the ERCC5

Reviewer 2

Major comments:
1. Bladder Cancer is a very prevalent disease and there would be no problem in getting the more samples. This type of polymorphic studies needs a large no of sample size but the sample size in this study is very small for getting any conclusive results.

   Answer to comment 1:
   I agree with you that the sample size in this study can be small for getting any conclusive results. However according to 3 reasons we think that our sample size is very representative to study the effect of gene repair polymorphisms in bladder cancer development:
   - First reason: this pathology was not very frequent in Tunisian population. Indeed only 200 subjects were annually diagnosed for bladder cancer in Tunisia.
   - Second reason: Tunisia is a very small country with less than 11 millions peoples
   - Third reason: the frequencies of studied alleles were high (more than 30%).

2. In Material & Methods: Is there any previous clearance from the ethical committee of Hospital?

   Answer to comment 2:
   This study was approved by a local ethical committee. We have added this information in “Material & methods” section.

3. In the results, Haplotype analysis and Linkage disequilibrium should be incorporated for more meaningful conclusion. Carriage rate should also be calculated and compared between both the groups.

   Answer to comment 3:
   I think that that is not important to do the Linkage disequilibrium test because the XPC, XPG and XPD gene were located in three different chromosomes, as so they are physically and genetically independents

4. Exclusion and inclusion criteria for the selection of both the groups are not properly discussed in Method section.

   Answer to comment 4:
   To clarify how controls were recruited we added this sentence in the material and methods section: “Controls were recruited daily from patients newly diagnosed and treated at the same urology department for benign diseases, mainly prostatic hyperplasia, cystitis and urolithiasis. Patients with cancer, or liver or renal diseases, were excluded”. Moreover we have indicated all information about
patients.

5. Discussion is not well written. Results should be discussed with other recent findings associated with bladder cancer.

Answer to comment 5: The results were discussed with other recent finding “see discussion section”

Minor comments:
1. In the introduction first and second paragraph, is reference less.
Answer to comment 1: I think that the paragraph 1 and 2 were well referenced
2. Fig is not reproducible
Answer to comment 2: There isn’t a figure in the text
3. In Table 5, Page 21 Sexe should replaced by Sex. The same correction for Table 4 also.
Answer to comment 3: We have replaced “Sexe” by “Sex”
Answer to comment 4: The statement, “Bladder cancer -------- base-excision repair pathway” statement is referenced

5. Methods, page 2: What "demographic information" was collected and how?
Answer to comment 5: Demographic information were: age, sex, geographic origin. We collect these information by asking for subjects (cases and controls)

6. Statistical Analysis, page 18: Please explain binary logistic regression. Was this technique used to determine odds ratios?
Answer to comment 6: Yes the logistic regression is used to determine the odds ratio indicated in the table by: “Exp(B)”. To explain the binary logistic regression we have added this paragraph in the materials and methods part: “The logistic regression is a mathematical modelling approach that is used to describe the relationship of several predictor variables X1, X2, ..., Xk to a dichotomous dependent variable Y which is typically coded as 1 or 0 for its two possible categories”.

Reviewer 3
Discretionary Revisions:
Comments 1
The authors have classified the study subjects into non-smokers and light smokers (1-19 PY); what was the basis for including 1-19 PY and not 1-10 PY or 1-15 PY as the criteria for light smokers.

Answer to comment 1:
We have classified subjects into non-smokers and light smokers (1-19 PY); our choice is supported by the recommendation of our epidemiologist. Indeed the mean of tobacco use in Tunisian population was 20PY.
Comments 2

2- In the Discussion, the authors have stated that the allele frequencies for XPC*C, XPD*C and XPG*C in the control group were estimated at 0.352, 0.331 and 0.326 respectively, and these frequencies were similar to that previously reported for Caucasians populations; the authors may specify the minor allele frequencies that have been previously demonstrated in Caucasians.

Answer to comment 2:

I agree with the reviewer and we have added this paragraph in the text “These frequencies were different to which reported for the Caucasian populations. Indeed, Goode et al. [24] have reported the frequencies of 0.38 and 0.23 for the XPD*C and XPG*C variants. In fact Mechanic et al. [25] and Agalliu et al. [26] have reported that the frequencies of XPC*C, XPD*C and XPG*C in control groups were respectively estimated at 0.39/0.48, 0.36 and 0.23/0.45.”

Comments 3:

Data presentation in Tables may be improved so as to make the presentation more impressive. The authors may consider indicating PCR product sizes in Table 1. Tables 3, 4 and 5 could be made precise and only relevant values upto 2 decimal places be indicated.

Answer to comment 3:

- The PCR products sizes were added in the table 1
- Values in tables 3 to 5 were corrected as suggested by the reviewer