Author's response to reviews

Title: Detecting 22q11.2 deletion in Chinese children with conotruncal heart defects and detection of single nucleotide polymorphisms in the haploid TBX1 locus in cases of 22q11.2 deletion

Authors:

Yue-juan Xu (xuyj1006@yahoo.com.cn)
Jian Wang (labwangjian@126.com)
Rang Xu (rang_xu@hotmail.com)
Peng-Jun Zhao (pjunzhao@sina.com)
Xi-Ke Wang (wangxike2008@sina.com)
Heng-Juan Sun (sunhengjuan@yahoo.com.cn)
Li-Ming Bao (lb_80220@yahoo.com)
Jie Shen (shenxiaomin@yahoo.com)
Qi-Hua Fu (ghuafu@hotmail.com)
Fen Li (lifeng_88@yahoo.com.cn)
Kun Sun (sunkunyh@yahoo.com.cn)

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Author's response to reviews: see over
July 7, 2011

Editor of
BMC Medical Genetics

Dear Editor,

We appreciated all the enlightening comments from the reviewers. The manuscript has been revised according to the comments. The modified parts were highlighted in blue. We also responded point by point to each reviewer comments as listed below. Thank you very much.

Best regards

Yours sincerely,

Corresponding author: Kun Sun, Ph.D, Professor
Department of cardiology, Xinhua Hospital
Shanghai Jiaotong University School of Medicine,
1665 KongJiang Road,
Shanghai 200092,
P.R China

Response to reviewer's comments:

Reviewer: Dana Crawford

Comments to the Author

1. The authors mention that a previous study conducted by the same authors found a lower prevalence of del22q11.2 in 24 Chinese CTD patients. Were these same 24 patients analyzed as part of the 212 patients reported in the manuscript? Either way, the authors should be explicit that the 212 either included or did not include the 24 previously examine patients.

Author's response:
The 212 patients enrolled in the study did not include the 24 previously examine patients. And we have changed them to “a group of new population of 212 patients” in revised manuscript. Please see the last paragraph of the Background section.

2. Under the subsection titled “Gene PCR and sequence”, several details are missing. For example, how were the polymorphisms identified (what software was used and with what settings)? For the controls, were any quality control metrics employed such as tests of Hardy Weinberg Equilibrium, etc? And, who are the healthy controls? No demographic data (sex, age, race/ethnicity, etc) is provided for these 139 dizygous healthy controls. Perhaps the demographic data for the controls can be provided in a table with the cases. And, why do the authors state that the healthy controls are needed to “confirm the results of the patients.” This statement does not make sense.
**Author’s response:**
We have made changes accordingly in revised manuscript. Please see the last paragraph of “Subjects”, the last sentence of the “Gene PCR and sequencing”, the “Statistical analysis” in the Methods section, and first paragraph of the “Gene PCR and sequencing” in the Results section.

3. In the same subsection, the authors state that “it may, therefore, be implied that the sister chromatid which contains the 933G is predisposed to be deleted.” This conclusion and statement is too strong based on the weak evidence of association (p=0.046). And, many tests of association were performed without correction or acknowledgement of multiple testing, so this is likely a false positive result.

**Author’s response:**
We have deleted this sentence in revised manuscript.

4. In the same subsection, the authors also state that “it is, therefore, possible that fetuses with del22q11 who carry the A allele in the rs41298838 locus were spontaneously aborted due to a flawed development of the heart.” Again, this statement is too strong given the very small number and weak statistical evidence.

**Author’s response:**
We have deleted this sentence in revised manuscript.

5. In the Discussion section, the authors state that “this may be due to the small size of our group investigated…”. Please include “(n=4)” in this statement to show the readers how small the sample size is.

**Author’s response:**
We have made changes accordingly in revised manuscript. Please see the second paragraph of “Discussion”. Thank you!

6. There are at least two statements in the Discussion section that are too strong based on the data collected and described here. The first is, “It, therefore, seems that either the sister chromatid, which contains the 933G allele in the locus, is predisposed to be deleted, or the fetuses carrying the G allele were miscarried during pregnancy due to unknown.” The second is, “It, consequently, is possible that del22q11 fetuses [sic] with the A allele in the rs41298838 locus were spontaneously aborted due to a faulty development of the heart or other relevant organs.” Also, the last two sentences of the section “Conclusions” are over-reaching given the small sample sizes and lack of power in this study.

**Author’s response:**
We have deleted the sentences in revise manuscript.

7. As mentioned above, the authors should include additional demographic data for the 139 control samples included in the study. Perhaps a new “Table 1” for all 212 patients and 139 controls detailing demographic data such as age, sex, etc could be included in the manuscript.
Author’s response:
We have added the demographic data for the 139 healthy controls in revised manuscript. Please see the second paragraph of the “Subjects”.

8. Table IV only displays the data for one SNP because it was significantly different in frequency between deleted patients and controls at $p=0.046$. Table IV should be expanded to include ALL tests of association so that readers are clear as to how many statistical tests were performed. The same comment applies to Table V: please show all the data for all tests of association performed.

Author’s response:
We have made changes accordingly and explained Table 4 and 6 in revised manuscript. Please see the part of “Tables”, the second paragraph of “Gene PCR and sequencing” in the Result section, the last paragraph of “Discussion”, and the third sentence of “Conclusion”.

9. In the Results section and subsection titled “Multiplex ligation-dependent probe amplification”, the authors state that the incidence is “lower than the potential microdeletions reported by authors in the West.” What are the references to these reported by “authors in the West”?

Author’s response:
We have added the reference “[2]” in revised manuscript.

10. In the Results section and subsection titled “Gene PCR and sequence”, the authors state that “all of the substitutions had a frequency of $p>0.05...$” This does not make sense—perhaps the $p$ (a symbol for p-value) is misplaced here?

Author’s response:
We have changed them for “a minor allele frequency (MAF) of $>0.05$” in revised manuscript. Please see the first paragraph of “Gene PCR and sequencing” in the Results section.

11. The x-axis of Figure 3B is too small to read.

Author’s response:
We have made changes accordingly in revised manuscript. Please see the Figures.

12. How do the control sample frequencies compare with the International HapMap data?

Author’s response:
The allele frequencies of SNPs observed in our study were similar to the HapMap Han Chinese. And we have explained it in the first paragraph of “Gene PCR and sequencing” in the Results section.

13. There are several minor typos throughout the manuscript. For example, there is a misplaced common in the Introduction. The authors sometimes italicize gene names, and they sometimes do not. “Chromarid” is used instead of “chromatid” in the Discussion section.
Author’s response:
We have revised the errors accordingly. Thank you!

Reviewer: Sintia Belangero

Comments to the Author

General comments:
Human gene names should be written in italic and all capital fonts (gene international nomenclature).

Author’s response:
We have made changes accordingly in revised manuscript. Thank you!

Title:
The word “discovery” should be changed for “detection”.

Author’s response:
We have made changes accordingly in revised manuscript. Thank you!

Abstract:
1) To define CTD acronym.
2) The authors have proposed to determine the pathogenesis of 22q11.2 del syndrome, however, no 22q11.2 del syndrome patient was included in this study. Only isolated conotruncal heart defects patients were recruited. To change pathogenesis of 22q11.2 del syndrome for pathogenesis of conotruncal heart defects.

Author’s response:
We have made changes accordingly in revised manuscript. Thank you!

Methods:
Unnecessary describing karyotype and MLPA protocols, since they are widespread and established techniques. This part can be drastically shortened. Subheading “Gene PCR and Sequence” should be changed for “Gene PCR and Sequencing”.

Author’s response:
We have made changes accordingly in revised manuscript. Thank you!

Results:
In the first line: The authors should add “numaerical”, e.g. “No obvious structural and numerical abnormalities”
Regarding to subheading MPLA:
1) The whole first paragraph should not be in “Results”. This is a method specification.
3) When describing the deletion size (for example, CLTCL1-LZTR1), to quote in Mb and to give respect to LCRs which are well-known references.

Regarding to subheading FISH:
1) The whole first paragraph should not be in “Results”. This is a method specification.
2) To indicate where the D22S75 marker is located on region map (figure 2).

Author’s response:
We have made changes accordingly in revised manuscript. Please see the section of
Results. Thank you!

**TBX1 Sequencing:**
To change “a frequency of p>0.05” for “a p value>0.05”
The statements “It may, therefore, be implied that sister chromatid…” and “Prior studies have found that although the TBX1…” should be in Discussion and not in Results.

**Author’s response:**
We have changed “a frequency of p>0.05”) for “a minor allele frequency (MAF) of >0.05” in revised manuscript, and deleted the sentences according to comments of Dana Crawford.

**Tables:**
Considering the tables III, IV and V, there are nine SNPs reported, whereas in the text eight are mentioned. The rs41298838 is not presented in table III. Is it a mistake typing?

**Author’s response:**
We detected 8 different sequence variants in the haploid TBX1 gene of the 13 del22q11 patients, and added Figure 4 to elaborate them. The 9th SNPs rs41298838 was found in both the non-del22q11 patients and healthy controls, however, only allele G was found in the locus of the haploid 22q11.2 in all of the 13 del22q11 patients.

**Figure 3:**
It is useless and adds nothing to the comprehension.

**Author’s response:**
We have deleted the Figure 3A and kept the 3B. Because Figure 3B explained the three types of deletion. Thank you!

**Other editorial requirements**

**Comments to the Author**

**Ethical Approval:** Can you please name the committee as you as authors have different affiliations.

**Author’s response:**
Our study was undertaken with the approval of the Medical Ethics Committee of Shanghai Children’s Medical center (SCMC). And we have made changes accordingly in revised manuscript. Thank you!

**Copyediting:**

**Author’s response:**
We have revised the errors and asked our colleague to correct the language. Thank you!

**The title page:**

**Author’s response:**
We have made changes accordingly in revised manuscript. Thank you!

**Abstract and Keywords:**

**Author’s response:**
We have made changes accordingly in revised manuscript. Thank you!

**Reference citations:**

Author’s response:

We have made changes accordingly in revised manuscript. Please see the section of “Reference”. Thank you!

**Tables:**

Author’s response:

We have made changes accordingly in revised manuscript, and added the subsection of “Assessment of clinical manifestations” for the Table 2. Thank you!

**Figures:**

Author’s response:

We have made changes accordingly in revised manuscript. Thank you!