PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between vitamin D receptor gene FokI polymorphism and skeletal fluorosis of the brick-tea type fluorosis: a cross-sectional case-control study.
AUTHORS	Yang, dan; Liu, Yang; Chu, Yanru; Yang, Qing; Jiang, Wei; Chen, Fuxun; Li, Dandan; Qin, Ming; Sun, Dianjun; Yang, Yanmei; Gao, Yanhui

VERSION 1 - REVIEW

REVIEWER	Zeliha KAYAALTI Forensic Sciences Institute, Forensic Toxicology, Ankara University
REVIEW RETURNED	06-May-2016

GENERAL COMMENTS	Study design is appropriate. In general, manuscript has been well written and easy to understand. This study and its results are so intelligent. I really liked fluent presentation of the study. Only in manuscript, there are some typing errors. They should be checked. Sometimes 3.2mg/L, sometimes 3.2 mg/L (with space) have been written. In Page 9, Line 158: “china” should be written “China”

REVIEWER	Giovanni Lombardi IRCCS Istituto Ortopedico Galeazzi, Milano, Italia
REVIEW RETURNED	11-May-2016

GENERAL COMMENTS	In this paper the authors analyzed the association between brick-tea consumption-associated skeletal fluorosis and FokI polymorphism in the VDR gene. Strenghts & Limitations - the authors reported the main findings of the work. Only one partial limitation is reported. Please use this section more appropriately Introduction - Please, better describe the pahtogenesis of skeletal fluorosis with a particular focus on its possible association with vitamin D Materials & Methods - there is no mention about the sample size. Please describe - In statistical analysis the power analysis calculation should be reported to justify the sample size - Skeletal fluorosis diagnosis should be briefly described Results - enumerate the risk factor used to correct analyses Discussion - the concept of CT/TT as a protective factors in Mongolian is repeated several times. Authors should discuss more deeply about the risk associated with CC genotype and about how this could be

	associated with the pathogenesis of fluorosis - Porbably measuring vitamin D serum concentrations would be helpfull in exploring pathogenesis Some senetences should be corrected: pag 9 lines 51-52 pag 14 lines 24-25 pag 14 "does" instead of "dose"

REVIEWER	Wajih Kaabachi Tunis El Manar University; Tunisia
REVIEW RETURNED	09-Jun-2016

GENERAL COMMENTS	- Please reduce the introduction. - Authors should provide more specific details in the discussion part and discuss more again theirs finding. - Is this a case-control study or a cross-sectional study? What's the differences and when we use each method and under any conditions? - Does only the presence of the FokI polymorphism is considered to be a regulator of bone metabolism and calcium resorption. Do rally acts only? Or the whole vitamin D receptor gene is implicated? What is the probable role of this SNP in the occurrence of this disease? - However, agreement on this relationship between is not universal among different populations. What do you think about this fact? - Please rewrite many sentences as 'this protective of CT/TT genotype in VDR-FokI appeared….,….'. or "Calcium could against the toxic effects of fluoride to a certain extent. - The authors say "Our data suggest that the CT/TT genotype of VDR-FokI may be a protective factor for the brick-tea". According to your results it is a protective factor factor or may be? Please clarify and specified the nature of this factor (molecular, chemical, and genetic…..). - Please in table 1 put the abbreviation for IF and UF - Total fluoride intake estimates (IF) do she includes dietary or non-dietary or both sources. Please clarify? - Why the author's choice to work with this genetic model? For the clarity of study, they should use the co-dominant genetic model in addition to the dominant model in the table 2. - What's the nature of gene environment interaction studied in this article? Please clarify and discuss them. - Why she use to work on the Fluoride excretion in the urine as a biomarker to predict fluoride intake? - An important role for the vitamin D was introduced in many studies around the world, highlighted these facts in relation to your findings.

VERSION 1 – AUTHOR RESPONSE

Replies to Reviewer 1 Only in manuscript, there are some typing errors. They should be checked.
Sometimes 3.2mg/L, sometimes 3.2 mg/L (with space) have been written.
In Page 9, Line 158: "china" should be written "China"
We appreciate your valuable suggestion. We have corrected these typing errors.

Replies to Reviewer 2
Strenghts & Limitations: the authors reported the main findings of the work. Only one partial limitation is reported. Please use this section more appropriately.

Thanks. We have added two limitations: 4.Our sample size of Tibetan and Han paticipants was inadequate, so the true association of VDR-FokI polymorphism with skeletal fluorosis in these two ethicities is worth to further study. 6. We did not have the data on the serum vitamin D.
Introduction - Please, better describe the pahtogenesis of skeletal fluorosis with a particular focus on its possible association with vitamin D
Thanks. We added the sentence “It is reported that fluoride modulates the VDR mRNA expression. [14]” at page 5 line 93~94.
Materials & Methods - there is no mention about the sample size. Please describe; In statistical analysis the power analysis calculation should be reported to justify the sample size.
Thanks. We added the sentence “Post-hoc power of the study was estimated using G*Power software (version 3.1).” at page 8 line 162~163.
- Skeletal fluorosis diagnosis should be briefly described
Thanks. We added the sentence “the diagnose of skeletal fluorosis was based on the sign of X ray, included osteoporosis, osteomalacia, sclerosis, ossification of soft tissue and joint degeneration in the forearm, shank, and pelvic, and could be classified into three categories: mild, moderate or severe.”at page 7 line 137~140.
Results
- enumerate the risk factor used to correct analyses
Thanks. We investigated the potential interactions between FokI-SNP and potential risk factor in Mongolian participants (Table3).
Discussion - the concept of CT/TT as a protective factors in Mongolian is repeated several times. Authors should discuss more deeply about the risk associated with CC genotype and about how this could be associated with the pathogenesis of fluorosis
Thanks. We added the sentence “Animal study indicated that excess fluoride had an inhibitory effect on duodenal VDR gene transcription, and thereby hindered the calcium absorption process.[34]” at page 12 lines 250~252, and “Moreover, evidence indicated that the TT and CT forms of the VDR-FokI polymorphsim are associated with a decreased VDR efficiency, compared with the TT genotype.[24]” at page 12 lines 254~256.
- Porbably measuring vitamin D serum concentrations would be helpfull in exploring pathogenesis
We appreciate your valuable suggestion. Unfortunately, we have no sample to detect serum vitamin D.
Some senetences should be corrected: pag 9 lines 51-52; pag 14 lines 24-25; pag 14 "does" instead of "dose".
Thanks. We have corrected.

Replies to Reviewer 3
- Please reduce the introduction.
Thanks. We have rewrote some patrs.
- Authors should provide more specific details in the discussion part and discuss more again theirs finding.
Thanks. We have rewrote some patrs.
- Is this a case-control study or a cross-sectional study? What’s the differences and when we use each method and under any conditions?
In this study, we first undertook a cross-sectional in sixteen villages from the Inner Mongolia, Qinghai and Sinkiang province, People’s Republic of China, from July to August in 2012 and recruited 1284 subjects. Then subjects with skeletal fluorosis were as case, others were as control.
- Does only the presence of the FokI polymorphism is considered to be a regulator of bone metabolism and calcium resorption. Do rally acts only? Or the whole vitamin D receptor gene is implicated? What is the probable role of this SNP in the occurrence of this disease?
Besides FokI, others SNPs, such as BsmI et al. also play an important role in bone metabolism. So it is required to study the association between these SNPs and skeletal fluorosis. These sentences were added at page 15 line 295~297.

- However, agreement on this relationship between is not universal among different populations. What do you think about this fact?
Given non-significant nature of association of VDR-FokI polymorphism in Tibetan, Kazakh and Han paticipants, it is necessary to estimate if our study has adequate power to detect the true association if present in these populations. We estimated the power of our study sing G Power software (version 3.1). We obtained the power of 98.9% and 99.4% at p = 0.05 for overall and Mongolian participants, respectively. But the power for Tibetan, Kazakh and Han paticipants is 11.7%, 64.5% and 8.1% respectively. This shows that our sample size of Tibetan, Han paticipants was inadequate and the study was insufficiently-powered to detect the true association of VDR-FokI polymorphism with skeletal fluorosis, if existent in these ethinic participants. These sentences were added at page 11 line 209~218.
- Please rewrite many sentences as 'this protective of CT/TT genotype in VDR-FokI appeared….,….'. or "Calcium could against the toxic effects of fluoride to a certain extent.
Thanks. We have corrected these sentences.
- The authors say "Our data suggest that the CT/TT genotype of VDR-FokI may be a protective factor for the brick-tea". According to your results it is a protective factor factor or may be? Please clarify and specified the nature of this factor (molecular, chemical, and genetic…..).
We have added sentences "FokI polymorphism, a C→T transition found within the translation initiation site in exon 2, results in long and short variants ofVDR due to creating an upstream initiation codon (ACG→ATG).[21] FokI polymorphism was related to bone mineral density and calcium absorption. [21-23] Some reports suggest that the short receptor protein (C allele), initiated from the second ATG site, may plays a more active role in the VDR responsive gene expression because it interacts more efficiently with the transcriptional factor IIB compared to the long receptor protein (T allele), initiated from the first ATG site.[24]" at page 6 lines 102~109.
- Please in table 1 put the abbreviation for IF and UF
Thanks. We did so.
- Total fluoride intake estimates (IF) do she includes dietary or non-dietary or both sources. Please clarify?
Thanks. It is intake of tea fluoride (ITF).
- Why the author's choice to work with this genetic model? For the clarity of study, they should use the co-dominant genetic model in addition to the dominant model in the table 2.
In our study, VDR-FokI polymorphism followes a dominant model of inheritance. This sentence was added at page 10 line 197.
- What's the nature of gene environment interaction studied in this article? Please clarify and discuss them.
It was discussed at page 13~14 lines 267~284.
- Why she use to work on the Fluoride excretion in the urine as a biomarker to predict fluoride intake?
Most fluoride is ecreted through urine, so urine fluoride is used as a biomarker to predict fluoride load.
- An important role for the vitamin D was introduced in many studies around the world, highlighted these facts in relation to your findings.
Thanks. Unfortunately, we have no data on the serum vitamin D.

VERSION 2 – REVIEW

REVIEWER	Giovanni Lombardi I.R.C.C.S. Istituto Ortopedico Galeazzi, Milano, Italia
REVIEW RETURNED	16-Aug-2016

GENERAL COMMENTS	All the requests have been addressed