Author's response to reviews

Title: A novel Bruton's tyrosine kinase gene (BTK) missense mutation in a Chinese family with X-linked agammaglobulinemia

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Author's response to reviews: see over
RESPONSES TO THE COMMENTS

<A novel Bruton's tyrosine kinase gene (BTK) missense mutation in a Chinese family with X-linked agammaglobulinemia>

Referee 1
Reviewer: Pamela Lee

Reviewer's report:
The authors reported a case of XLA with classical presentation. The case is straightforward without diagnostic issues. A novel BTK mutation was identified and its pathogenicity is analyzed by bioinformatics tools.

Minor Essential Revisions
1. BTK, when referring to the gene, should be in italics
2. Page 2, line 43: 'that' is duplicated
3. Page 3, line 58: ‘increased transparency of the bilateral lobes’ - authors please specify the finding with standard radiological term
4. Page 3, line 59: should be Streptococcus pneumoniae in italics
5. Page 3, line 61: lymphadenectomy should either be replaced by lymphadenopathy or lymphadenitis, whichever is appropriate
6. Page 3, line 66: the abbreviations of blood biochemistry are not standard and should be given in full (HBDH, TP, A, G)
7. Page 3, line 68: give reference range for ferritin
8. Page 3, line 72: delete the word 'suspected'
9: Page 3, line 86: the affected family member is a cousin, not a sibling according to the family tree. And the individuals in the family tree should be labelled with the corresponding number in each generation (I, II, III)
10. Page 5, line 114: specify incidence as live births or live male births
11. Page 5, line 128: specify unit of molecular mass. The standard unit is kilodalton (kDa)

We have corrected all above minor essential revisions in the manuscript.

Discretionary Revisions
1. Page 2, line 34: 'The incidence of XLA is the highest among the PIDs' - this is probably not true, now with universal newborn screening for SCID the collective incidence of SCID probably exceeds that of XLA. This piece of information is not essential to this report and can be omitted.

We have omitted it in the manuscript.

2. Screening of BTK mutation for the patient's family members was performed and male relative (III-2) was found to be affected. Is he symptomatic? How old is this relative (cousin) when BTK mutation was found? What is his immunological profile (Ig G/A/M, % B-cells)? It would be interesting if this cousin has mild or relatively milder symptoms so that he was not previously investigated, and adds importance to genetic confirmation of BTK mutation in
family screening and diagnosis of affected family members. 

*The male relative has not obvious severe infectious symptoms, even if the boy had some mild infections such as URI his patients did not pay more attention to this.* This is a 1-year-old boy when he was found with a mutation of BTK gene and the test of immunoglobulins and B cell counts were performed at the same time. We have added the result in the manuscript. The boy was not previously investigated for the two major reasons: the first is that he is lack of symptoms of severe infections; the second is that the family comes from a remote area and their poor economic conditions couldn’t support their medical test fees. Surely, some patients with XLA have clinical presentation very late even more than 10 years old late. We have illustrated the importance of gene tests on the diagnosis of the affected family members in the conclusion part.

3. The administration of IVIG described by the authors is suboptimal for the management of patients with XLA. IVIG is recommended to be given 3-4 weekly, and dosage should be adjusted according to the trough level and clinical symptoms.

Reference: Use of intravenous immunoglobulin in human disease: A review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology, J Allergy Clin Immunol 2006;117: S525-53

Sure, according the reference you mentioned the standard IVIG replacement therapy is recommended to be given every 1 month. But the drug of immunoglobulin is very expensive and the current medical insurance system in China does not cover it for the patients with XLA. So we administered IVIG replacement to the patient every 4 weeks at the beginning of the first 3-months, then prolonged intervals of administration to 2 or 3 months. We monitored the level of immunoglobulins before administration and sustain it above 5g/L to prevent the patient from infections. We also adjusted the dosage and intervals based on the clinical symptoms in the follow up.

4 The most comprehensive database for BTK gene variations is the BTKbase, Version 8.53 last updated 17 June, 2013 contains 1254 public entries. This should be mentioned in addition to Human Gene Mutation Database. 

http://structure.bmc.lu.se/ijdbase/BTKbase/index.php?content=index/IDbases

We have mentioned it in the manuscript.

Referee 2

Reviewer: SHYH-DAR SHYUR

Reviewer’s report:

The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

There are some comments regarding this paper that should be pointed out.

1. In P1, L16 - the sentence “X-linked agammaglobulinemia (XLA) is a rare inherited disease characterized by recurrent viral and bacterial infections” should be rewritten, because viral infections are usually handled normally with the exceptions of hepatitis viruses and enteroviruses.
We deleted the ‘viral infections’ from the manuscript because the major etiology of infection for the patient with XLA is recurrent bacterials.

2. In P1, L23-24 – about the sentence “A genetic analysis of the family revealed an affected male sibling with a c.1117C>G mutation”, the author should detail the clinical manifestations and management of this affected male sibling. This male sibling patient has not server infections. We have added the result of immunological portrait and therapy in the manuscript. The detail result of blood works had been illustrated in the ‘genetic analysis’ part already.

3. P2, L42-43 – the sentence “Any mutation that that occurs in any site within one these 5 domains” should be corrected to “Any mutation that occurs in any site within one of these 5 domains” We have corrected it in the manuscript.

4. P2, L51-53 – the sentence “In the subsequent 2 years, the boy experienced 1 episode of septicemia, 3 episodes of otitis media, 2 episodes of pneumonia, and 4 episodes of bronchitis.”, The author should detail the causative specific pathogens of each episodes. We treated the septicemia of this patient in our hospital and the blood culture and sputum culture had been done at the time. But the bronchitis and otitis were diagnosed and treated in other primary hospital and the etiology of infection was unkown, perhaps not done. We have added the known pathogens in the manuscript.

5. P6, L142-147 – In patient with initial IgG < 0.34 g/L, he received IVIG 400 mg/kg/month for the first 3 months followed by subsequent infusions once every 2-3 months. The author should detail the follow-up serum IgG concentration. We have added the detail concentration of serum IgG in the manuscript.

Reviewer 3
Reviewer: Melinda Erdos
Reviewer’s report:
Authors have described a novel BTK missense mutation in a Chinese family with X-linked agammaglobulinemia. The language of the manuscript is reasonable.

Major Compulsory Revisions:
1. Paragraph “Case Presentation”, line 71: it would be better to give the absolute count of CD19+ B-lymphocytes rather than the percentage. We have added the value of CD19 B cell absolute count in the manuscript.

2. Figures 1 and 2 have to be modified into one figure. Electropherograms have to be showed under the family tree, and have to be indicated by the number of the family member (eg: “III.1” for patient, etc; and “C” for control). Symbols used in the family tree (Figure 2) are completely surprising. The authors should take into account the standardized human
pedigree nomenclature and symbols. In a case of an X-linked disease, affected males should be indicated with solid squares (instead of a half shaded square), and female carriers should be indicated with a circle that has a dot inside (instead of a half shaded circle). In the II. generation of the family tree husbands are completely unnecessary to indicate and number, so the list in Paragraph “Genetic analysis”, line 87 correctly is the following: (I.1, II.1, II.2, II.4).

*We have modified Figures 1 and 2 into one figure and corrected the family tree.*

3. The literature is not appropriately cited. All but one reference were published before 2005. It would be important to refer newly published papers; especially those which based on cohort studies give comprehensive overview of the molecular genetic and demographic features of XLA.

*We have cited some newly published papers in the manuscript.*

Questions:

4. What was the method of the genetic analysis of BTK gene?

*The entire coding region of the BTK gene was amplified by polymerase chain reaction and analyzed by direct sequencing.*

5. What was the clinical history of the affected other sibling in this family?

*The affected 1-year-old male sibling had not severe infectious symptoms with the exception of mild URI. His blood work showed he had a low count of circulating CD 19+B cell and Hypogammaglobulinemia. We had added the detail clinical and laboratory portrait into the part of genetic analysis in the manuscript.*

6. Are there other disease-causing nucleotide changes in position c.1117 described before?

*There is no other disease-causing nucleotide changes in position c.1117 described before.*

7. The patient receives IVIG substitution only in every 2-3 months, rather than in every 4-6 weeks, as in XLA patients it used to. What is the reason for that?

*The standard IVIG replacement therapy is recommended to be given every 1 month. But the drug of immunoglobulin is very expensive and the current medical insurance system in China does not cover it for the patients with XLA. So we administered IVIG replacement to the patient every 4 weeks at the beginning of the first 3-months, then prolonged intervals of administration to 2 or 3 months. We monitored the level of immunoglobulins before administration and maintain it above 5g/L to prevent the patient from infections. We also adjusted the dosage and intervals based on the clinical symptoms in the follow up.*

8. Did the authors perform any assay to predict the functional consequences of the novel mutation at protein level?

*We will perform assay at protein level in the further experiments.*

Minor Essential Revisions:

9. In the text Figure 1A is not mentioned.

10. Paragraph “Introduction”, line 43: “that” is
repeated

We have corrected it in the manuscript.

11. Paragraph “Case Presentation”, line 51: “anti-viral” is not needed
We have corrected it in the manuscript.

We have corrected it in the manuscript.

13. Paragraph “Case Presentation”, line 59: it is not clear what “(+++)” means after streptococcus pneumonia
It is impertinence to illustrate the quantity of cocc pneumonia with “(+++)' so we had deleted the “(+++)’from the manuscript according to your sugesstion.

We have corrected it in the manuscript.

15. Paragraph “Case Presentation”, line 69: “immunodeficiency” is not needed
We have corrected it in the manuscript.

16. Paragraph “Discussion”, line 134: “in” is repeated
We have corrected it in the manuscript.

17. Paragraph “Abbreviations”, line 165: the abbreviation of allophycocyanin is missing and it is also not found in the text
We have omitted it in the line 165.

18. Paragraph “Abbreviations”, line 165: the abbreviation “IVIG” is not for “gammaglobulin replacement therapy”
We have change it to intravenous immune globulin

Referee 4
Reviewer: Jer-Ming Chang
Reviewer’s report:
Major Compulsory Revisions
In the “Discussion”, authors chiefly described what are mostly already known. However, the discussion related with the analysis itself is lacking. I suggest that authors may consider discussing the cost-effectiveness of such an analysis, especially if it concerns the future treatment with IVIG. Also, readers would like to realize if IVIG is the only way of treatment and how about the effect of IVIG in other reports? Are there other treatment options? Please discuss some more content about the past publication on this topic.
We have completed more discussion on the therapy of XLA in the ‘discussion’ part of the manuscript according to the suggestions.
Minor Essential Revisions

1. The first sentence of the Introduction: “X-lined ……. is the first primary immunodeficiency disease (PID) to be identified”. I wonder why this rare disorder is the first PID to be identified? In which condition? Please explain or rephrase. Or, the “to be” should be omitted?

   We have changed the ‘first primary PID’ to ‘the major PID’ and omitted the ‘to be’ in the manuscript.

2. The second paragraph of the Introduction: “one these 5 domains”. Should it be “one of these 5 domains”? Also, according to my recollection, the five domains should be PH, TH, SH3, SH2, and TK, but not SH1. Please verify.

   We have corrected it in the manuscript.

3. In the Case Presentation, authors mentioned the name of Streptococcus pneumonia. As a general rule, the name of the bacteriae should be italicized (oblique).

   We have corrected it in the manuscript.

4. In the last paragraph of Discussion: “The serum IgG levels were also monitored and maintained above 5 g.” Please explain what does it mean to maintain IgG above 5g?

   When the serum IgG concentration is above 5g/L, it will be efficiency to keep patient away from infections.

5. What is the clinical status of individual III-2? Is he also an XLA patient? There should be 2 males being affected in this family, thus in page 5, line 140, a total should be 2 males.

   We have corrected it in the manuscript.

6. In page 5, line 134 " in the in exons" should be "in the exons"

   We have corrected it in the manuscript.

7. In Figure 1: Please consider to put DNA chromatograms A, B, and C in a row and adding the amino acids on top of them for better expression.

   We have corrected it in the manuscript.

8. In Table 1: Formal binomial nomenclature should be used, like H. Sapiens for human.

   The nomenclature of different species in table 1 is according to ensemble genome browser (http://asia.ensembl.org/index.html). We insisted that there was little reason to change it.

9. The index case is very young. Authors may have to provide the description of the vaccination records

   The parents could only provide the history of BCG immunization for their child. For the recurrent infections the patient stops all the programmed vaccination in the follow up.