Author's response to reviews

Title: Atypical X-linked agammaglobulinaemia caused by a novel BTK mutation in a selective immunoglobulin M deficiency patient

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Version: 4 Date: 26 August 2013

Author's response to reviews: see over
Dear BMC Pediatrics Editorial Team,

Thank you for your kind editorial letter of 07/27/2013.

We have now responded point-by-point to the editorial board and all 4 Reviewers’ requests. Please let us know if our answers fulfill reviewers’ questions for consideration of *BMC Pediatrics* publication.

Thank you very much for your consideration.

Sincerely,

Daw-Yang Hwang MD, PhD

Aug. 25, 2013
Response to Reviewers

Reviewer's report (1)

Title: Atypical X-linked Agammaglobulinemia caused by a Novel BTK Mutation in a selective IgM Deficiency Patient.

Version: 3 Date: 9 June 2013

Reviewer: Esko Wiltshire

Reviewer's report:

Lim et al proved an interesting case report suggesting the phenotype of XLA may be broader than previously suspected. The background and case report are generally well written, and well referenced.

Case report: How was the haematuria/proteinuria originally detected? Were there symptoms? Routine screening? Other reasons?

Author reply:
His hematuria and proteinuria were detected during a routine urine screen. The case did not have symptoms related to his non-nephrotic range proteinuria. Accordingly, we now changed the following sentences in the main text (page 6):
“A 6-year-old Chinese boy with a 2-year history of persistent hematuria and proteinuria found by routine screen was referred to our department.”

Most importantly, what evidence do the authors have that the c.347C>T (p.P116L) mutation is pathogenic and not a polymorphism? They need to provide this to justify their conclusions, and without this information it is difficult to determine whether the case report adds new information.

Author reply:
We agree that the functional aspect of this c.347C>T (p.P116L) BTK is important to differentiate a pathological mutation versus a rare variant. At preparation of this rebuttal letter, we are unable to obtain fresh blood from the patient to provide further information suggested by the reviewer. However, we tried to provide some evidences that c.347C>T (p.P116L) is probably a pathological mutation.

Accordingly, we now added the following sentences in the main text (Page 8-9):
“Amino acid P116 is well conserved among different species (Figure as attachment). Variant c.347C>T was not observed in the BTKbase (http://bioinf.uta.fi/BTKbase/), HGMD Biobase (http://www.biobase-international.com/product/hgmd), 1000 Genomes Projects (http://www.1000genomes.org/), and NHLBI Exome Sequencing Project (ESP)( http://evs.gs.washington.edu/EVS/). The direct sequencing of 100 individuals from Taiwan of Chinese origin from Taiwan was performed and no c.347C>T variant
was detected (data not shown). Computer estimations of the function of p.P116L are labeled “disease causing” by MutationTaster (http://www.mutationtaster.org) and “probably damaging” (HumDiv score of 0.998 and HumVar score of 0.949) by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), respectively. Further analyses, including the expression of BTK in mononuclear cells from the patients and alteration in the tyrosine 223 phosphorylation in monocytes after activating via Toll-like receptors, should provide the degree of functional defect of this mutation.”

**Minor Points:**

1. Page 5 line 9 [pleksin]
2. Page 5 line 12 “defective B cell developments” + development
3. Page 6 fourth to last line – Kidney Ultrasound?
4. Page 7 final lie should be” diagnoses” not diagnosis
5. Page 10 3 last line “maintained” should read “remained”

**Author reply:**
Accordingly, we now changed all above mentioned typos/errors in the main text.
Reviewer’s report (2)

**Title:** Atypical X-linked Agammaglobulinemia caused by a Novel BTK Mutation in a selective IgM Deficiency Patient.

**Version:** 3  **Date:** 21 June 2013

**Reviewer:** Maria Marluce S Vilela

**Reviewer’s report:**

The article writes about a missense mutation type (p.P116L), which occurs when one amino acid is substituted for another. Being a missense mutation and not yet published in the literature, I think the authors should do additional testing to prove that it is not polymorphism but a new mutation in BTK. Authors can seek nucleotide exchange (c.347C>T) in healthy controls by enzyme restriction or direct sequencing. If this same exchange occurs in healthy controls, it is polymorphism. I cannot say what would be the ideal number of controls to be analyzed, but we suggest evaluating 100 controls. But some investigators analyze around 800 controls.

**Author reply:**

In accordance with reviewer’s suggestion, we performed direct sequencing on 100 individuals from Taiwan of Chinese origin. No c.347C>T variant was found in these 200 chromosome.

Accordingly, we now added the following sentences in the main text (page 8-9):

“Amino acid P116 is well conserved among different species (Figure as attachment). The variant c.347C>T was not found in the BTKbase (http://bioinf.uta.fi/BTKbase/), HGMD Biobase (http://www.biobase-international.com/product/hgmd), 1000 Genomes Projects (http://www.1000genomes.org/), and NHLBI Exome Sequencing Project (ESP)( http://evs.gs.washington.edu/EVS/). The direct sequencing of 100 individuals of Chinese origin from Taiwan was performed and no c.347C>T variant was detected (data not shown).”

<Figure>

Homo_sapiens
Mus_musculus
Rattus_norvegicus
Gallus_gallus
Xenopus_tropicalis
Danio_rerio
Homo_sapiens_ITK
Homo_sapiens_IEC

P116
To complement the analysis, there are also computer programs (Mutation taster, Polyphen 2) to predict the pathogenic potential of mutation.

Author reply:
In accordance with reviewer’s suggestion, we performed computer analysis of this c.347C>T variant.
Accordingly, we now added the following sentences in the main text (page 8-9):

Computer estimations of the function of p.P116L are labeled “disease causing” by MutationTaster (http://www.mutationtaster.org) and “probably damaging” (HumDiv score of 0.998 and HumVar score of 0.949) by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), respectively.
Reviewer’s report (3)

**Title:** Atypical X-linked Agammaglobulinemia caused by a Novel BTK Mutation in a selective IgM Deficiency Patient.

**Version:** 3  **Date:** 10 July 2013

**Reviewer:** GUHA KRISHNASWAMY

**Reviewer's report:**

**Major revisions:**

This is an interesting patient with presumed XLA and recurrent sinopulmonary infections who developed nephropathy.

1. **The statement that low IgM requires evaluation for XLA and btk mutation is erroneous and a generalization that should not be suggested by a single case report. The near normal IgG levels may represent a leaky phenotype.**

   **Author reply:**

   We believe our case represent a leaky phenotype of XLA. We agree that a case report cannot apply to the general low IgM population, especially for those individuals with secondary IgM deficiency. However, familial cases of selective IgM are very rare and deserve further study. Accordingly, we now changed the following sentence in the main text (P.3):

   “We suggested that B lymphocyte surface antigen studies and BTK mutation analysis should be performed in familial patients of selective IgM deficiency to exclude atypical XLA.”

   And in the main text (P.12):

   “Two patients in an IgM deficiency study exhibited equal to or less than 2% CD19+ B cells, indicating that XLA should be a differential diagnosis in familial selective IgM deficiency despite normal IgG level, as in our patient.”

2. **The use of IVIG infusion for slgM deficiency as suggested by this manuscript should not be a recommendation as it is impossible to reconstitute IgM deficiency with IVIG for obvious reasons. This needs to be clarified**

   **Author reply:**

   We agree the IVIG should not be given routinely, but should be given in cases of recurrent, debilitating or life threatening infection, and/or in patients with concomitant functional IgG deficiencies as previously suggested (Goldstein 2008 Clinical and Developmental Immunology 2008:624850).
Accordingly, we now changed the following sentence in the main text (P.12): “Currently, no molecular defect has been determined to be responsible for IgM deficiency, and IVIG may be instituted in cases of recurrent, debilitating or life threatening infection, and/or in patients with concomitant functional IgG deficiencies.”

3. I wonder why a sinus CT was not done or results discussed

**Author reply:**

Sinus CT was not done. The sinusitis was proven by Water’s view which showed bilateral obliterated maxillary sinuses with mucosal thickening.

4. What was the reason for the very elevated IgE in the brother?

**Author reply:**

The brother had atopic dermatitis history, which may be compatible with the elevated IgE. Accordingly, we now changed the following sentence in the main text (P.6): His family history was unremarkable except that his elder brother, who had experienced recurrent sinusitis and atopic dermatitis, had been diagnosed with selective IgM deficiency at the age 3 years.

5. A good evaluation of antibody responses to pneumococcal and/or DT immunization is required to confirm functional defect- this has not been provided

**Author reply:**

Unfortunately, we do not have data concerning the abovementioned antibody titer on these 2 siblings.

6. Could the low B cells result from immunosuppressive therapy?

**Author reply:**

Immunosuppressive therapy, like steroid, do decreases lymphocyte numbers and immunoglobulin productions. However, immunosuppressive therapy usually affect both B and T lymphocytes (except for target therapy), and lower only CD19+ B cell to equal or less than 1% is impossible. We do not think immunosuppressive therapy is the cause of low B cell count.
7. **What is the 24 hour urine analysis result on this patient? Could the nephropathy have been a post-infectious complication (such as seen with PSGN)?**

**Author reply:**

1) The 24 hours urine analysis showed daily protein loss of 1.4 g/day. Accordingly, we now added the following sentences in the main text (page 7): “His daily protein loss was 1.4 g/d.”

2) We agree with reviewer’s point regarding PSGN and actually PSGN was one of the differential diagnoses of this patient at the beginning. Several episodes of upper respiratory infection with low C3, CH50, and deposition of IgG and C3 in the kidney biopsy make PSGN a differential diagnosis. However, these were no “acute episode” of edema, hypertension, gross hematuria or elevation of serum creatinine. Antistreptolysin-O titer was not elevated with any prominent endocapillary proliferation or neutrophils infiltration under light microscopy. Thus we were unable to make PSGN as our diagnosis.

8. **Dosage of IVIG used for vasculitis and autoimmunity are different from dosages used as replacement therapy for PID- there is some confusion regarding this**

**Author reply:**

In our patient, IVIG was used due to increase frequency of sinusitis and for treatment of his proteinuria. To clarify this, we change the sentence in the main text (page 7): Because of increased episode of infections and persistent proteinuria, the treatment regimen was followed by IVIG of 400 mg/kg/ 4 wk for a total of 16 weeks with no change in his proteinuria.”
9. English language needs to be reviewed throughout the manuscript

Author reply:

This manuscript has been sent to a professional English academic editing center for further review (Please refer to attachment for certificate)
Reviewer’s report (4)

Title: Atypical X-linked Agammaglobulinemia caused by a Novel BTK Mutation in a selective IgM Deficiency Patient.

Version: 3 Date: 12 July 2013

Reviewer: Leopoldo Santos-Argumedo

Reviewer’s report:

*The phenotype of the case presented in this article is very interesting because it is a selective IgM deficiency with normal levels of IgG and IgA. In addition, the patient presented autoimmunity, described as "Lupus-like Nephritis" in the abstract (which is not described as such in the rest of the manuscript). The authors detected immune complexes in a kidney biopsy, and the patient was treated with several immunosuppressive drugs, apparently succeeding with mycophenolate to treat the autoimmunity.*

Author reply: Accordingly, we now changed the following sentence in the main text (P.7):

“These lupus-like pathology results were inconsistent with his clinical and autoimmune profile, whereby the diagnosis of systemic lupus erythematosus cannot be made.”

*The manuscript describes a clinical phenotype that is totally different from what is known of XLA. The only data that correlates with XLA is the low proportion of B cells in peripheral blood; however, other humoral deficiencies (such as common variable immunodeficiency) can be also manifested with low levels of B cells. The incidence of autoimmunity in XLA is very low, and rheumatoid arthritis is the autoimmune manifestation most often associated with XLA. The description of Lupus-like phenotype is very interesting and I may recommend its publication. Unfortunately for the authors, this "mutation" has not been described by any other group, what remains to be determined without a doubt is whether this is actually a pathological mutation and not a polymorphism. There is no additional data that support the idea that this mutation affects the expression of Btk in mononuclear cells from the patients, or perhaps there is no information about the functionality of mutated BTK, assessed for example as alteration in the phosphorylation (tyrosine 223) in monocytes after activating via TLRs. Any of these data, or even better, both of them, could contribute to the hypothesis that the genetic change found is actually a mutation affecting the expression and/or activation of Btk and therefore its functionality.*
Author reply:

We agree that the functional aspect of this c.347C>T (p.P116L) *BTK* is important to differentiate a pathological mutation versus a rare variant. At preparation of this rebuttal letter, we are unable to obtain fresh blood from the patient to provide further information suggested by the reviewer. However, we tried to provide some evidences that c.347C>T (p.P116L) is probably a pathological mutation.

Accordingly, we now added the following sentences in the main text (Page 8-9):

“Amino acid P116 is well conserved among different species. Variant c.347C>T was not found in the BTKbase (http://bioinf.uta.fi/BTKbase/), HGMD Biobase (http://www.biobase-international.com/product/hgmd), 1000 Genomes Projects (http://www.1000genomes.org/), and NHLBI Exome Sequencing Project (ESP)(http://evs.gs.washington.edu/EVS/). The direct sequencing of 100 individuals from Taiwan of Chinese origin was performed and no c.347C>T variant was detected (data not shown). Computer estimations of the function of p.P116L are labeled “disease causing” by MutationTaster (http://www.mutationtaster.org) and “probably damaging” (HumDiv score of 0.998 and HumVar score of 0.949) by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), respectively. Further analyses, including the expression of BTK in mononuclear cells from the patients and alteration in the tyrosine 223 phosphorylation in monocytes after activating via Toll-like receptors (Vargas-Hernandez, et al, *IUMBM Life* 2012 64:346), should provide the degree of functional defect of this mutation.”

Moreover, few studies have also shown decreased levels of IgM, with normal IgG; for example, a paper from Basile N., et al (*J Clin Immunol* 2009 29:123–129) describes a patient (#12) from a cohort of 49 XLA patients, having normal levels of IgG with decrease IgM. However, the mutation found in this patient is very frequent in XLA and this patient does not have autoimmunity. In summary, it seems to me that this very interesting clinical phenotype must be accompanied by a clear demonstration of loss of function of Btk in order to sustain the finding.

Author reply:

We agree that the functional aspect is important as mentioned, especially this c.347C>T maybe a hypomorphic mutation (suggested by the clinical phenotype). It is believed that other genetic and environmental factors might affect the diverse phenotypes of XLA.

Accordingly, we now added the following sentences in the main text (page 9):

“Normal levels of IgG accompanied with decrease IgM have been reported in other cases involving BTK mutation in XLA (Basile et al, *J Clin Immunol* 2009;29:123), but no autoimmune diseases have been reported.”