Author’s response to reviews

Title: Comprehensive chromosomal aberrations in a case of a patient with TCF3-HLF-positive BCP-ALL

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Author’s response to reviews:

Lublin February 28th 2020

Dear Editor-in-Chief of the BMC Medical Genomics,

Please find enclosed the revised manuscript MGNM-D-20-00025: “Comprehensive chromosomal aberrations in a case of a patient with TCF3-HLF-positive BCP-ALL” by Monika Lejman, et al., to be submitted as a Case Report to the BMC Medical Genomics for consideration of publication.

All co-authors have seen and agree with the contents of the manuscript and declare no conflict of interest. We certify that the submission is original work and is not under review at any other publication.

As compared to the first submission, the revised version of the manuscript contains additional details prepared according to Reviewers comments (Figure 1, Figure 2, Figure 3 and Figure 4). The results were also described in more detail. The manuscript was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at Springer Nature Author Services (verification code: A5B8-80EA-48A6-4EB7-6A20).

We believe that our findings could be of interest to the readers of the BMC Medical Genomics, because the raised issues still are very popular among clinicians. We hope that the editorial board will agree on the interest of this study.

I would like to thank you for your comments.
Below I indicate the revisions, which were made according to Reviewer’s comments. The changes are highlighted.

Reviewer # 1:

This report describe a rare patient with a deletion of a long arm fragment of chromosome 13 (13q12.2-q21.1) and co-existing TCF3-HLF fusion. I still have several questions.

Comment 1:

Have you process CytoScan HD (Affymetrix) microarray for copy number variation (CNV) analysis on the patient’s parents? It is better that the hereditary feature is thorough, then we can tell if the CNV is resonable for ALL.

Revision and my comment

The patient's parents were examined and their molecular karyotypes were correct. Several genetic factors are associated with an increased risk of ALL (e.g. Down syndrome). Moreover, specific polymorphic variants in particular genes (e.g. IKZF1, GATA3, ETV6) may indicate an increased risk of ALL or a specific subtype of this disease [1]. However, these factors are not common enough to be routinely analyzed in patient’s pedigree. In this case, we would like to analyze if translocation t(17;19) is balanced. Sometime when we analyze breakpoints, we notice unbalanced deletion in breakpoint regions. Our patient had a lot of clinical complications during the first line of therapy and drug resistance occurred in relapse treatment. Given the fact that we are dealing with an extremely rare case, performing additional analyzes would be an undeniable advantage of our work.

Comment 2:

Can you tell us whether RB1, PAX5, NOTCH1, CDKN2A and CDKN2B is dominant or recessive pathogenic genes? If some genes are recessive pathogenic genes, further whole exon sequencing or whole genomic sequencing maybe necessary to confirm another allele.

Revision and my comment

RB1 displays autosomal dominant inheritance, while the mutant RB1 gene acts as an autosomal recessive gene at the cellular level. RB1 is a suppressor gene, thus the mutation in both alleles determines the development of retinoblastoma [Berry JL, Polski A, Cavenee WK, Dryja TP, Murphree AL, Gallie BL. The RB1 Story: Characterization and Cloning of the First Tumor Suppressor Gene. Genes (Basel). 2019; 10(11): 879.]. For the PAX5 gene, inherited mutations have not yet been described. However, rare germline mutations in PAX5 and ETV6 are linked to

Unfortunately, the number of samples collected from the patient was not sufficient for further analyzes, including whole genomic sequencing. The 13q deletion was found in the samples at diagnosis but it was not observed in samples from relapse, which indicates the clonal evolution of cancer cells. The correct karyotype of patient’s parents does not indicate the necessity for additional analysis of their genetic material.

Comment 3:

Is there any evidence that could prove fusion gene TCF3-HLF and the long arm fragment of chromosome 13 (13q12.2-q21.1) are related to poor outcomes?

Revision and my comment

TCF3-HLF fusion is associated with poor prognosis, as highlighted in the manuscript (Background section, lines 63-67, page 4; Discussion and Conclusions section, lines 128-133, pages 6-7), citing papers [2,3,4,6].

In the case of an isolated 13q deletion, outcome depends, among others, on the size of the lost chromosome fragment. Based on the relationship between karyotype and phenotype, patients with 13q deletion with or without RB1 deletion are classified into specific groups. Patients with a deletion proximal to 13q32 (group 1) show mild to moderate mental retardation, variable dysmorphic features and growth retardation. Patients with deletions extending into 13q32 (group 2) show one or more major malformations including severe microcephaly, and malformations of the brain, genitourinary and gastrointestinal tract [Mitter D, Ullmann R, Muradyan A, et al. Genotype-phenotype correlations in patients with retinoblastoma and interstitial 13q deletions. Eur J Hum Genet. 2011;19(9):947–958. doi:10.1038/ejhg.2011.58 ]

In our case, the patient did not require genetic consultation, the symptoms mentioned above did not occur in her case.
The 13q deletion was observed in the patient's genetic material collected at the time of diagnosis, but this change was not found in the material collected at relapse. The loss of the RB1 gene in a patient with TCF3-HLF fusion was described by us for the first time.

Reviewer # 2:

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

Revision and my comment

All comments were provided according to the Reviewers requests. The manuscript was reorganized.

Reviewer # 3:

This case report presents a rare BCP-ALL case with TCF3-HLF fusion combined with additional chromosomal variations. The case itself holds clinical significance, providing insights to the understanding and possible treatment of the disease. In the current version of the manuscript, the relationship between TCF3-HLF and the additional variations are not illustrated thoroughly. Perhaps a different journal would be more appropriate for the article. Still, there are a few points whose revision could increase the value and clarity of the publication for the reader:

Comment 1:

Page 4, line 53: A reference backing up the incidence percentage would be nice.

Revision and my comment

The reference [1] was provided (Background section, line 60, page 4).

Comment 2:

Page 4, line 69-70: Perhaps a rephrase to "No central nervous system involvement was observed" would be better, as the original statement may mislead the readers to think there was, at that time, some undiscovered central nervous system involvement.
Revision and my comment

The sentence was corrected (Case presentation section, lines 78-79, page 4).

Comment 3:

Page 4, line 70-74: Results of the FISH tests demonstrating a lack of chromosomal aberrations (both at initial treatment and relapse), if available, should be provided. Also, as is shown in figure 2a, the deletion of 13q12.2-q21.1 would be possible to be observed in a karyotype examination, would it not? Therefore, the statement "the karyotype was normal" seems questionable, and may need a brief explanation.

Revision and my comment

The results of the FISH tests at initial treatment and relapse were provided (Figure 1 and Figure 2) (Figure description section, lines 359-378, pages 16-17). The absence of aberrations of 13q in the karyotype analysis indicates that only cells with a normal karyotype have grown in cell culture and we have referred to this issue in manuscript (Case presentation section, lines 113-118, page 6). Deletion of 13q was confirmed on interphases by FISH (del 13q14 in diagnosis - 86,4%; CCP13/CCP21 FISH Probe Kit, Cytotest).

Comment 4:

Page 5, line 80: "Clinic", the capitalization doesn't seem to be necessary.

Revision and my comment

The sentence was corrected (Case presentation section, line 92, page 5).

Comment 5:

Page 5, line 85: Do you mean that the myelogram presented 75% blasts? Perhaps a rephrase is needed.

Revision and my comment

The sentence was corrected (Case presentation section, lines 100-101, page 5).

Comment 6:
Page 5, line 91-98: What is the percentage of blasts in which the 13q deletion, the PAX5 and NOTCH1 variations took place, respectively? Are they limited to the same cell population with the presence of TCF3-HLF fusion?

Revision and my comment

We have referred to this issue in the manuscript (Case presentation section, lines 113-118, page 6). The 13q deletion and TCF3-HLF fusion were present in the same cell clones. The results obtained in the recurrence testify to the clonal evolution of cancer cells because no 13q deletion was found, while the percentage of blasts with TCF3-HLF fusion was 77%. In our laboratory, we do not have probes enabling the visualization of changes within NOTCH1 and PAX5. The analysis of NOTCH1 and PAX5 alterations was possible only due to the microarray technique.

Comment 7:

Following point 6. Readers would be interested in the relationship of these genetic variations, and may raise questions such as: Do they always occur in the same subgroups of cells in this patient? Does one type of the variations make the cell more prone to other variations? At what stage did the fusion/deletion/duplication take place, simultaneously or not? A short discussion of the above aspects would be interesting for the reader, even if these questions cannot be answered definitively.

Revision and my comment

We have referred to this issue in the manuscript (Discussion and Conclusions section, lines 183-187, page 9; lines 214-218, page 10). Watanabe et al. suggested that TCF3-HLF fusion may be accompanied by other genetic changes. Among the most commonly described changes co-occurring with the TCF3-HLF fusion are alterations in the RAS signaling pathway genes (NRAS, KRAS), as well as in the VPREB1 and BTG1 genes. In our case, no changes in any of these genes were observed. However, the PAX5 deletion associated with the TCF3-HLF fusion was found both in our patient and in the previously described cases. The 13q deletion was observed in the patient's genetic material collected at the time of diagnosis, but this change was not found in the material collected at relapse. The loss of the RB1 gene in a patient with TCF3-HLF fusion was described by us for the first time.

Comment 8:

Page 6, line 102: A reference backing up the poor prognosis would be nice. Or maybe line 105-106 could be moved here to avoid repetition.

Revision and my comment
The paragraph was corrected (Discussion and Conclusions section, lines 128-135, pages 6-7, ref. [4,5,6]).

Comment 9:

Page 7, line 131-136: In this patient, is the RB1 deletion restricted to the blasts? Are there any abnormalities concerning chromosome 13, or RB1, in the patient's pedigree? As the loss of 13q is of great significance to not only this work but to the field as well, a deeper discussion of the possible mechanisms lying behind would be beneficial.

Revision and my comment

We have referred to this issue in the manuscript (Discussion and Conclusions section, lines 183-187, page 9).

Comment 10:

Relating to the discussion part of the article: The manuscript presents a thorough review of each of the genetic anomalies in the patients. However, readers would be more interested in the situation where all these anomalies lie in one patient. A discussion of the relationship of these genetic variations is highly recommended. What is their relationship? How did they act interactively to cause this patient's situation?

Revision and my comment

We have referred to this issue in the manuscript (Discussion and Conclusions section, lines 214-218, page 10).

Comment 11:

The figures have poor resolution. If improved, it will greatly satisfy the readers.

Revision and my comment

The figures have been corrected (Figure 1, Figure 2, Figure 3, Figure 4).

Comment 12:
Fig 1b: Type I of the fusion is difficult to understand. Where did EX16 go? Which part is the "insertion" composed of? Perhaps it would be easier to understand with more thorough figure legends.

Revision and my comment

This figure has been improved and appropriate description has been provided (Figure 4, lines 399-409, page 18).

Comment 13:

Fig 2: A brief annotation/legend will help a lot for better understanding of this figure.

Revision and my comment

This figure and its description has been corrected (Figure 3, Figure description section, lines 389-398, page 18).

Reviewer # 4:

Within the manuscript "Comprehensive chromosomal aberrations in a case of a patient with TCF3-HLF-positive BCP-ALL", the authors describe a single clinical case of translocation t(17;19)(q22;p13), using FISH probing and microarray analysis. They postulate that identification of additional genomic aberrations beyond the gene fusion event may inform on the clinical course of the disease.

Whilst the study presents an interesting medical case, there are several gaps in the study approach that require addressing to support the conclusions. There are also several key issues relating to the manuscript that also require elaboration or amendment.

Comment 1:

As a general comment, could I suggest that the authors seek manuscript editing help from someone with full professional proficiency in English? At times, the manuscript was difficult to follow, and I think improving the written communication would assist with conveying the study effectively

Revision and my comment
The manuscript was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at Springer Nature Author Services (verification code: A5B8-80EA-48A6-4EB7-6A20).

Comment 2:

For ease of reading, I've broken the manuscript comments into each relevant section, and included line numbers where appropriate.

Abstract: The abstract does not clearly state why this case is novel or why it should be reported. The authors note that the TCF3-HLF fusion has already previously been reported in the literature, albeit in a small number of cases. Though aberrations in RB1, PAX5 and NOTCH1 are further diagnosed in the study patient, the cumulative effects or implications (diagnostic or prognostic) are not stated.

Revision and my comment

As suggested, we highlighted the novelty of our work and justified why it should be reported (Abstract, Background section, lines 38-40, page 2; Abstract, Conclusions section, lines 49-53, page 3). Our results, illustrating comprehensive changes in the karyotype and clonal evolution of cancer cells, call into question the effectiveness of experimental therapy. The cumulative effect of the described changes has both diagnostic and therapeutic implications. The patient was treated in accordance with the ALLIC-BFM 2009 protocol, according to which the stratification of patients is based on a karyotype analysis and the presence of BCR/ABL1, KMT2A, ETV6/RUNX1 rearrangements. The updated version of this protocol (AIEOP-BFM ALL 2017), introduced in Poland in 2018, imposes the obligation to diagnose TCF3-HLF fusion. The described case is another step to understand the complexity of the impact of other changes on the outcome of patients with established TCF3-HLF fusion. Additional research is necessary to determine the mechanism by which comprehensive changes can affect the outcome.

Comment 3:

Background: (Lines 55 and 56) Examples of chromosomal rearrangements, SNVs and Indels resulting in ALL should be listed and referenced here. Otherwise, these are generic empty statements.

Revision and my comment

According to the Reviewer’s comment, this statement was deleted as it's not necessary to understand the case.
Comment 4:

Case Presentation:

(Line 71) Abbreviations such as FISH should appear in the parentheses, with the full definition preceding this.

Revision and my comment

The sentence was corrected (Case presentation section, line 81, page 4).

Comment 5:

(Lines 73 and 74) If the patient karyotype was determined by GTG band staining, the authors should show this as a figure to confirm normality. If the statement of a normal karyotype is solely based on the observed FISH results, this would only be suggestive of a lack of disruption at these select loci, and not overall patient normalcy.

Revision and my comment

We have referred to this issue in the manuscript and provided the patient’s karyotype (Case presentation section, lines 113-118; Figure 1A, Figure description section, lines 359-368, pages 16-17). The absence of aberrations of 13q in the karyotype analysis indicates that only cells with a normal karyotype have grown in cell culture. Deletion of 13q was confirmed on interphases by FISH (del 13q14 in diagnosis – 86.4%; CCP13/CCP21 FISH Probe Kit, Cytotest).

Comment 6:

(Lines 84 and 85) The authors should provide definitions for HIA block and HIB block.

Revision and my comment

The names HIA and HIB quoted in the text are the names of therapeutic blocks, which include specific combinations of drugs. The terms given are not abbreviations but the names of chemotherapy treatment course. According to the Reviewer’s suggestion, we have provided the description of these courses in the manuscript (Case presentation section, lines 97-104 page 5).

Moreover, to avoid confusion, we have added the information about HIA and HIB blocks in the list of abbreviations (List of abbreviations section, lines 235-238, page 11).
Comment 7:

(Lines 88 to 98) Out of all the retrospective tests that could be applied, why specifically was TCF3-HLF FISH performed? If it was part of a battery of FISH tests, what other assessments were attempted?

Revision and my comment

Performing additional analyzes of patient's genetic material is a routine procedure used in patients with short survival in whom a very aggressive disease course and resistance to treatment were observed. Following the data discussing cases of patients with a similar course of the disease, we used probes for TCF3-HLF.

Comment 8:

(Lines 91 to 96) Whilst the microarray was applied to samples at diagnosis and at relapse, TCF3-HLF FISH (and GTG band staining) only seems to have been applied at diagnosis. Why is this? Further, it would also be more comprehensive for the authors to show the effects of genomic disruption of RB1, PAX5, CDKN2A, CDKN2B and NOTCH1 through either qRT-PCR methods or IHC. I.e. show an effect on each respective gene at either the transcriptional or translational level. qRT-PCR would also provide good confirmation of the TCF3-HLF FISH result, both at diagnosis and at relapse.

Revision and my comment

We have referred to this issue in the manuscript (Case presentation section, lines 119-120, page 6). Given the fact that we are dealing with an extremely rare case, performing additional analyzes would be an undeniable advantage of our work. Unfortunately, the amount of protected samples from the patient was not enough for further tests, including RT-PCR.

Comment 9:

Discussion and Conclusions: (Lines 108 to 110) A reference is needed for TCF3.

Revision and my comment

The references [7,8] have been provided (Discussion and Conclusions section, line 138, page 7).

Comment 10:
What elements of each gene are incorporated into the new fusion gene, and what are the implications of such a composition?

Revision and my comment

We have referred to this issue in the description of Figure 4 contains information about the TCF3-HLF fusion (Figure 4; Figure description section, lines 399-409, page 18). Moreover, we added the information about TCF3-HLF fusion into the manuscript (Discussion and conclusions section, lines 141-154, page 7).

Comment 11:

A reference is needed for the well-established deletions of PAX5, VPREB1 and BTG1.

Revision and my comment

The reference [3] has been provided (Discussion and Conclusions section, line 156, page 8).

Comment 12:

"Demonstrated" is a little strong here. Mouse models don't always faithfully recapitulate human disease. "Implied", or similar, may be a better substitute.

Revision and my comment

The sentence was corrected (Discussion and Conclusions section, line 161, page 8).

Comment 13:

Figures: (1A) There is no description of the figure labelling or figure colours in the legend.

Revision and my comment

The figure was improved and the description has been provided (Figure 1 and Figure 2; Figure description section, lines 359-378, pages 16-17).

(1B) Some of the details of this figure legend would be better served in the main body of the text. The figure itself is of very poor quality, is not well annotated and is barely legible.
Revision and my comment

The figure was improved (Figure 4, Figure description section, lines 399-409, page 18). Moreover, we added the information about TCF3-HLF fusion into the manuscript (Discussion and conclusions section, lines 141-154, page 7).

(2A+B) The legends for both figures lack any descriptive detail. The figures also appear to be low-quality screen snapshots, with poor labelling and unclear annotation.

Revision and my comment

The figure was improved (Figure 3) and its description has been provided (Figure description section, lines 389-398, page 18).

Sincerely yours,

Monika Lejman on behalf of the authors.

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