Author's response to reviews

Title: Mutations in the STK11 gene in Czech Peutz-Jeghers families

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Author's response to reviews: see over
To
Matthew Kaiser PhD, Assistant Scientific Editor
on behalf of
Scott Edmunds PhD, Senior Scientific Editor

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Re: MS: 1484620702251881

Dear Prof. Kaiser,

I am sending you the revised version of our manuscript entitled “Mutations in the \textit{STK11} gene in Czech Peutz-Jeghers patients”. We revised the manuscript according to the suggestions made by the referees. You will find enclosed the revised version of our MS and a letter addressing the comments and suggestions raised by the referees. We would be happy to hear from you soon.

Sincerely yours,

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Enclosures:
- revised manuscript
- cover letter giving a point-by-point response to the comments and suggestions made by the referees
Reply to the comments of the referee

First of all the authors would like to express many thanks to the referee for his/her review of the MS

Reviewer: J R Choi

Minor Essential Revisions:

We have revised all the spelling mistakes (as suggested)

Section Material and methods has been described in more detail including a note to follow manufacturer’s guide where necessary.

Section Patients has been included in the Material and methods as suggested.

List of abbreviations has been appended behind section Results and discussion.

Figure legends have been modified in more descriptive mode.
Reply to the comments of the referee

First of all the authors would like to express many thanks to the referee for his/her review of the MS

Reviewer: D Nageshwar Reddy

Minor Essential Revisions:

We have modified the statement in paragraph 7 in the Results and discussion “Konishi et al. reviewed 103 literature PJS patients …. was 31.2 years compared to duodenal carcinoma 39.7 years …..” as suggested.

Discretionary revisions:

1. We have redescribed the legends to chromatogram in more detail. Axis X- represents the lengths of the amplified fragments and axis Y- represents the relative PCR fragment (exon, intron) dosage where the specific probes are hybridized and compared to control sample.

2. We have updated the case C-1 description in section Patients (which has been moved below section Materials and methods) and modified the eighth paragraph in section Results and discussion.

Material and methods

Patients

“At the time of the molecular analysis of her genomic DNA for the STK11 germline mutation, another capsulated endoscopy examination was performed. A few diminutive (less than ten) polyps in the stomach and one polyp in the ileum were found. The polyp from the ileum showed characteristic histological features of adenoma. Polyps from the stomach were not biopsied.”
Reply to the comments of the referee

First of all the authors would like to express many thanks to the referee for his review of the MS

**Reviewer:** Rodney J. Scott

Major compulsory revisions:

We agree with the reviewer’s comments that there are other conditions that predispose to hamartomatous polyposis but in PJS there are some distinct features that delineate this disease from the others. We have added a new paragraph to Introduction.

“There are some differential syndromes of PJS which could be misdiagnosed. The pigmentation of the perioral region is an external hallmark of PJS and is not present in other hamartomatous polyposis syndromes which include Cowden syndrome CS (OMIM 158350), Bannayan-Riley-Ruvalcaba syndrome BRRS (OMIM 153480) and Juvenile polyposis syndrome JPS (OMIM 174900). Laugier-Hunziker syndrome LHS is another differential diagnosis of PJS characterized by benign melanotic pigmentation of the oral cavity and lips, associated with spotted macular pigmentation of the fingerprints and longitudinal melanonychia. The LHS is known to be benign disease without gastrointestinal polyposis and with no systemic manifestation [23].”


We have also updated and filled in histopathological information for cases B-1, B-2, B-3 and C-1. We were unable to find out neither additional information involving GI finding nor histology about hamartomas in other patients.

We also agree with the referee that there are other genetic conditions associated with buccal freckling as is Leopard syndrome (OMIM 151100), McCune-Albright syndrome (OMIM 174800) and Neurofibromatosis type 1 (OMIM 162200) but other features of these conditions and particular pigmentation of PJS probands make these conditions unequivocal. Therefore we did not mention these conditions in the manuscript and do not think they could be confused with PJS. We mentioned only a non genetic condition, Laugier-Hunziker syndrome, which was described in Introduction.

Section Material and methods has been described in more detail including a note to follow manufacturer’s guide where necessary.
Reply to the comments of the referee

First of all the authors would like to express many thanks to the referee for his review of the MS

**Reviewer:** Justo Lorenzo Bermejo

**Major compulsory revisions:**

We have changed the title of the article “Mutations in the *STK11* gene in Czech Peutz-Jeghers families”

The new title is:

“Mutations in the *STK11* gene in Czech Peutz-Jeghers patients”

Abstract: The sentence “Molecular analysis could be helpful in disease management of PJS probands.” was modified to “Molecular analysis can definitely confirm the diagnosis of PJS probands and could contribute to elucidation of possible genotype-phenotype association.

Methods: We have revised section Methods and hope it is more comprehensive now

“We investigated genomic DNA of 8 individuals from five Czech families by sequencing analysis of the promotor and the entire coding region including the splice-site boundaries of the *STK11* gene and by multiplex ligation probe-dependent amplification (MLPA) assay.”

We have also revised section Results and Conclusion to fit changes made in Abstract

Introduction:

We have added information about elevated risk of cancer in PJS patients. It was included into the first paragraph in Introduction. There are also several references to more elaborated articles related to cancer risk in PJS.

“The cumulative risk of all cancers in PJS patients by the age of 60 years is 60% and is increased ~8-fold, compared to general population [5].”


Patients:

We have added information about fulfillment of PJS criteria to the Table 1

Last sentence on page 3
We suppose the referee meant the sentence “In one individual, a presumptive diagnosis of PJS was made due to a first-degree relative with PJS and the presence of mucocutaneous hyperpigmentation.” The section Patients has been included in the Material and methods and the sentence has been changed to “In one individual, we made a presumptive diagnosis of PJS due to a first-degree relative with PJS and the presence of mucocutaneous hyperpigmentation.”

Results and discussion:

The sentence “All eight patients except one (A-2) underwent endoscopic procedures to examine the whole GIT.” was moved to section Patients, to the first paragraph as suggested.

It is possible to establish some relationship between genotypes and phenotypes?
Due to a small group of patients we are not able to establish any relationship between genotypes and phenotypes of our probands. According to literature and our reports we have hypothesized about relationship between genotype and phenotype in some patients with gastric cancer. It is described in section Results and discussion in the eighth paragraph.

We have changed the sentence “Members of family B (case B-1, B-2, and B-3) had almost identical clinical symptoms with decreasing age of onset of the first symptoms and detection of polyps” to “Members of family B (cases B-1, B-2, and B-3) had almost uniform clinical symptoms with variable age of onset of the first symptoms and detection of polyps.”

“two individuals without family history who do not fulfill PJS criteria were included in the study on the basis of the result from case C1 (?)”
Case C-1 was analyzed first, before admitting cases D-1 and E-1. Positive molecular analysis of the STK11 gene in C-1 proband forced us to analyze also these sporadic cases.

What was the aim of the discussion on GI cancer development in PJ patients?
We have tried to point out that there are few reports with gastric cancer which usually develops at an early age and has worse course then other types of cancers in PJS. There should be paid more attention to patients with molecularly confirmed PJS, especially those who have polyposis of the stomach.