Author's response to reviews

Title: Novel Deletion Alleles Carrying CYP21A1P/A2 Chimeric Genes In Brazilian Patients With 21-Hydroxylase Deficiency

Authors:

Fernanda B Coeli (fbcoeli@bol.com.br)
Fernanda C Soardi (soardi@unicamp.br)
Renan D Bernardi (renandarin@gmail.com)
Marcela de Araújo (maraujo@boldrini.org.br)
Luciana C Paulino (luciana.paulino@ufabc.edu.br)
Ivy F Lau (mpmello276@hotmail.com)
Reginaldo J Petroli (rpetroli@unicamp.br)
Sofia HV Lemos-Marini (sofia.lemos@terra.com.br)
Maria TM Baptista (terma@uol.com.br)
Gil Guerra Jr (gileandrea@uol.com.br)
Maricilda Palandi de Mello (mmello@unicamp.br)

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Author's response to reviews: see over

To
Scott Edmunds PhD
The BioMed Central Editorial Team
e-mail: editorial@biomedcentral.com
Tel: +44 (0) 20 3192 2013

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Dear Dr. Edmunds,

This is to re-submit the manuscript entitled “Novel Deletion Alleles Carrying CYP21A1P/A2 Chimeric Genes In Brazilian Patients With 21-Hydroxylase Deficiency” to be considered for publication in BMC Medical Genetics.

The manuscript has been revised to be submitted. We have addressed reviewer’s criticisms to version 2 of the manuscript as follow:

REVIEWER 1:

1) The authors should clarify to which group of patients or population the "9%" of chimeric A2A1P genes refer.

This percentage was obtained based in two different studies in Brasil and both references are cited in the manuscript:

2) The authors give ratios of the different probe-signals in MLPA (2:1 or 3:2) and it is not clear to this reviewer, how the confidence intervals for the different MLPA-probes have been determined.
We added in Experimental Section the following:

“Data were analysed using free Coffalyser MLPA data analysis software [33]. Normalized relative values ranging from 0.8 to 1.2 corresponded to two gene copies in the genotype, whereas values below 0.8 and above 1.2 corresponded to deletion (1 gene copy) and duplication (3 gene copies), respectively. This confidence interval was established by data obtained with five bimodular controls in each MLPA assay.”

The final normalization of data included the normalization of results for all probes within each sample and the normalization with results obtained with five controls (included in each assay) that had been genotyped before as having bimodular alleles. This resulted in the range described in the manuscript.

3) What is the meaning of instead in the sentence "...novel and rare mutations, respectively, instead of.." (abstract, results).

We eliminated “instead” and rearranged the phrase as follows: “Another allele was identified with a CYP21A1P/A2 gene carrying both p.P34L and p.H62L mutations in exon 1, however p.P30L, the most frequent pseudogene-derived mutation in this exon, was absent.”

4) Referring to and addressing founder effects, HLA-haplotypes would have been of interest in the carriers of deletions and chimeric genes.

As HLA-haplotypes were not available for the study, we considered C4/SNPs haplotypes similarly to other studies, such as:


5) In general, the authors should try to shorten the different sections, particularly the "background", and to focus the discussion. The impact of the study for research as well as potential implications for the patient and the respective therapy should be addressed and specified.

We shortened Background, Methods and Results and added some discussion on genotype-phenotype correlation (Discussion, page 14).

6) It would be helpful for reading of the "Results" section that the latter could be more structured - e.g. according to patients' numbers or other criteria.

We re-organized Results section to present data according to patients’ numbers from 1 to 20.
7) What do the authors suggest concerning the methods applied: MLPA and southern blotting in all cases, or is MLPA sufficient in addition to sequence analysis?

We changed the discussion (page 15) on this subject to:

“Our study showed that the combination of Southern blot and ASO-PCR/direct sequencing with MLPA tests may constitute an option for mapping and better characterize chimeric genes on RCCX monomodular alleles, especially in populations with high allelic diversity such as that in Brazil. MLPA has been proposed as a candidate with good potential to be used in neo-natal screening and in pre-natal diagnosis because it can be performed with very low amount of DNA [55]. Eventually, MLPA may substitute time-consuming Southern blot in cases were HCA diagnosis is urgent as it managed to estimate the CYP21A1P/A2 borders in almost all cases. However, to distinguish between deletions and large gene conversions in genetic studies searching for detailed allelic information, it would be more informative if more C4 and CYP21 probes were included in the analysis.”

Quality of written English: Not suitable for publication unless extensively edited

English has been revised.

REVIEWER 2:

Comments:

Most of the information is already present in literature, in particular data regarding MLPA (methods section) result repetitive. The paper is very long and the reading is heavy. The authors should provide the news in a simple and clear way. The novel chimeric gene bearing both p.P34L and p.H62L rare mutations is the real news in this paper and this discovery can be resume in a brief report or short communication.

Answer:

I general, we have shortened Background, Methods and Results. However we did not transform the manuscript to a brief report or short communication because we consider that it contains more important data than only the novel chimeric gene bearing both p.P34L and p.H62L rare mutations. Actually, it reports allelic variability within a population that had not been described before. Within this context, the manuscript also highlights that we have to be careful in interpreting MLPA results. Although it was informative for most cases, if it was not used in combination with other techniques some results would be misinterpreted.
We thank you for your attention.

Yours sincerely,

Maricilda Palandi de Mello, Ph.D.