Author's response to reviews

Title: Frequency of LCT -13910C>T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups

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Response to Reviews
MS: 9052505912802573 - Frequency of C/T-13910 single nucleotide polymorphism associated with adult-type hypolactasia/normolactasia among Brazilians of different ethnic groups

Reviewer: Torbjörn K Nilsson
Reviewer's report:
Terminology is the major problem with this paper. I noted the following usages which I think should be changed:
1. The recommended nomenclature for mutations should be followed: LCT -13910C>T is the name of the studied polymorphism according to Antonarakis& Den Dunnen's rules. Already in the title of the paper this should be changed, and throughout the paper.
2. The word "rate" should not be used to indicate how common an allele is, "frequency" or prevalence should be used. (Already in Abstract).
3. "Normolactasia" as opposed to hypolactasia should not be used. Lactase persistence (LP) is the natural expression, and is now widely used in research papers. (Already in Abstract).

We changed terminology in the title of the paper “Frequency of LCT -13910C>T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups” and throughout the manuscript.

Instead of “rate” we used allelic frequency, genotype prevalence and hypolactasia prevalence.

4. The statement of the Conclusion in Abstract is not very interesting, we expected hypolactasia to be very common in Brazil so it is nothing new. The real news here is that Browns had equal prevalence of the T allele, this is very surprising!
In my view, an interesting conclusion should be like this: "The lactase persistence allele, LCT -13910 T, was found in about 43% of both White and Brown Brazilians, but was absent among all Japanese Brazilians studied."

We accepted the suggestion and changed the conclusions: The lactase persistence allele, LCT -13910 T, was found in about 43% of both White and Brown and 20% of the Black Brazilians, but was absent among all Japanese-Brazilians studied.

Reviewer: Monica Mottes

Major compulsory revisions
The experimental scheme of the paper is very simple, nevertheless my major criticism is about the small sample size (<50) of two among the four groups tested.
Black Brazilians.
17 individuals can not be considered as representative of a highly heterogeneous population, as the Brazilians of African descent are. I would recommend that at least 50 individuals are genotyped, before allele frequencies are calculated.

**Japanese Brazilians**
I would recommend that at least 50 individuals are genotyped, before allele frequencies are calculated. The authors did not find any T-13910 allele within a total number of 68 (i.e. frequency <0.015). By extending their sample to 100 alleles they might be able to confirm this negative finding and therefore conclude that the -13910 polymorphism is not present in this ethnic group. By definition a locus is said to be polymorphic in a given population when the minor allele frequency is at least 0.01. Since the T-13910 allele is not present in Orientals, this finding would suggest very low interbreeding between Japanese-Brazilians and European-Brazilians groups.

We invited more Black and Japanese-Brazilians to enter the study, reaching a total of 50 Blacks and 53 Japanese-Brazilians.

**Black Brazilians.**

In the genotyped sample, the T-13910 allele shows a frequency =0.12. This is far too high for being of African origin, as the authors state in the Discussion (first paragraph). The T-13910 allele is very rare, if not absent, in most African groups with the only exception of pastoralist Fulani (Ingram et al., Hum Genet 2007; 120:779). Most likely, the T-13910 allele found by the authors within their Black Brazilians sample, is of European origin and is the outcome of genetic admixture, a consequence of extensive interbreeding with Europeans occurred through the centuries (Goncalves et al., Human Heredity 2008, 65:23). **Discussion should be modified accordingly.**

We modified the Discussion as follows: Black-Brazilians have the LCT-13910T variant of lactase persistence although at a low frequency. However, most likely the LCT-13910T allele of European origin is the outcome of genetic admixture, a consequence of extensive interbreeding with Europeans that occurred through the centuries [10].

.... all Japanese-Brazilians had LCT-13910 CC genotype, suggesting very low interbreeding between Japanese-Brazilians and European-Brazilians groups;

**Reviewer:** Dóra Nagy

**Major Compulsory Revisions**
1. The study is valuable because data about the Brazilian population in this field are scarce. Although the total number of 515 individuals is impressive, the total number of Black (17) and Japanese-Brazilian group (34) is not very representative and sufficient.
We invited more Black and Japanese-Brazilians to enter the study, reaching a total of 50 Blacks and 53 Japanese-Brazilians.

2. The Brazilian population is very diverse and the present inhabitants have many different ancestors (Amerindian, African, Portuguese, Italian, German, Spanish, Polish, etc.). It would be interesting to investigate the ancestry of the studied individuals with regard to the possible differences in the prevalence of hypolactasia in these groups. The prevalence of hypolactasia in groups with different ancestors could be compared with previous results in the literature. For example: the prevalence of C/T-13910 variants among Italian descendants compared to the study results of Italian research groups.

It is not possible due to the intense genetic admixture since 1500 when Portuguese first arrived in Brazil. According to Gonçalves et al. (Human Heredity 2008, 65:23-32) taking Brazil as a whole, 28% of individuals self-declared as White carried mtDNA African haplogroups and 33% had mtDNA Amerindian haplogroups.

3. In Methods, authors mention that symptomatic and asymptomatic individuals were included in the study. It would be important to give more specific information about the two groups (number of each group, age, sex distribution, symptoms, other diseases) and compare their results separately, as well.

When we mentioned symptomatic individuals were the ones referring bloating and flatulence; however, none associated their symptoms with milk ingestion.

4. Were clinical investigations (such as lactose hydrogen breath test, or measuring dissacharidase activity of intestinal samples or any other tests) carried out beside the DNA test? Did these individuals complete questionnaires on their milk drinking habits? The comparison of the results of the DNA tests and clinical, nutritional data would give us more precise information about the real prevalence of adult-type hypolactasia.

We have published already in Clinical Biochemistry 41 (2008) 628–630: “Single nucleotide polymorphism C/T-13910, located upstream of the lactase gene, associated with adult-type hypolactasia: Validation for clinical practice”. In this report we used hydrogen breath test as standard and found high agreement with the genetic test for lactase non-persistence. As the genetic test is less cumbersome we decided to use it to make a survey in the population.

5. As the authors mention, the C/T-13910 polymorphism is associated with hypolactasia in European but not in most of the African populations, where three other SNPs are identified to be associated with lactase persistence. That’s why the prevalence of C/T-13910 variants in Black and maybe Brown populations is not informative about the prevalence of hypolactasia/normolactasia. It is advisable to test the other SNPs in Black and Brown groups and compare the results with those of clinical investigation and data from literature.
Our further study will be analysis using DNA sequence in order to ascertain that other polymorphisms are associated with lactase persistence in African Brazilians and Japanese and also perform the hydrogen breath test; even though, in our routine work at the lab for the clinical practice, African Brazilians and Japanese have shown correlation between the results of genetic test and hydrogen breath tests. In our previous report, 12 patients were African Brazilians and we observed association of the results of both tests.

6. Table 1 and 2 show no different data, but their two different presentations. These data should be presented in one table.

We presented both Tables in one.