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Short Note

"Normal" Mice Have Normal Telomeres

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Abstract: In a recent article, Smoom et al.[1] identified an important amino acid variation in the helicase RTEL1 of the Algerian mouse (*Mus spretus*). Since the latter has telomeres five times shorter than the laboratory mouse (belonging to the *Mus musculus* species), they introduced this variation in laboratory mice, in order to obtain an engineered strain with human-length telomeres. Succeeding in doing this, they established the "Telomouse" strain. Here I demonstrate that, in reality, *Mus spretus* does not show this variation: the key amino acid is conserved in all rodent species, which all have human-length telomeres (including wild *Mus musculus* species). This finding in no way compromises the value of the research of Smoom et al. and the utility of the Telomouse strain. Rather, it raises the question of how the telomere length of *Mus musculus* (as a species) should be considered and the important consequences it has on studies on inter-species comparison.

Keywords: evolution; helicase; inbreeding; RTEL1; telomere

Reading the abovementioned article[1], I was disappointed by the idea (which is shared by most researchers) that *Mus musculus* has very long telomeres. In reality, this a characteristic of "classical" laboratory strains (Figure 1). Nonetheless, since the authors successfully reached their aim, I was surprised by the following contradiction: laboratory mice show the conserved character of having methionine 492 (M492) in RTEL1, but have not the conserved character of "short" (15-20 kb) telomeres, while *Mus spretus* has not the conserved M492, but has the conserved character of short telomeres. So, I checked for RTEL1 in all other *Mus* species and subspecies (identifying new orthologs in species for which only genome assemblies are available) and found that M492 is conserved in all species (Figure S1 in Supplementary material). Finally, I used *Mus spretus* genome assemblies to predict *de novo* the protein sequence and I found a surprise: RTEL1 in *Mus spretus* has M492 (Figure 2). Then I found that also the sequence present in the Ensembl genome database (www.ensembl.org) is identical to the one predicted by me (Figure S1 in Supplementary material). Because of all these findings, it can be said that *Mus spretus* protein sequence of RTEL1 (deposited by Ding et al.[2] and used by Smoom et al. in their article[1]) in the GenBank database shows a wrong amino acid in position 492. Thus, the amino acid 492 of RTEL1 is not responsible for the difference in telomere length between laboratory mice and *Mus spretus* (as well as between laboratory mice and all other mice).

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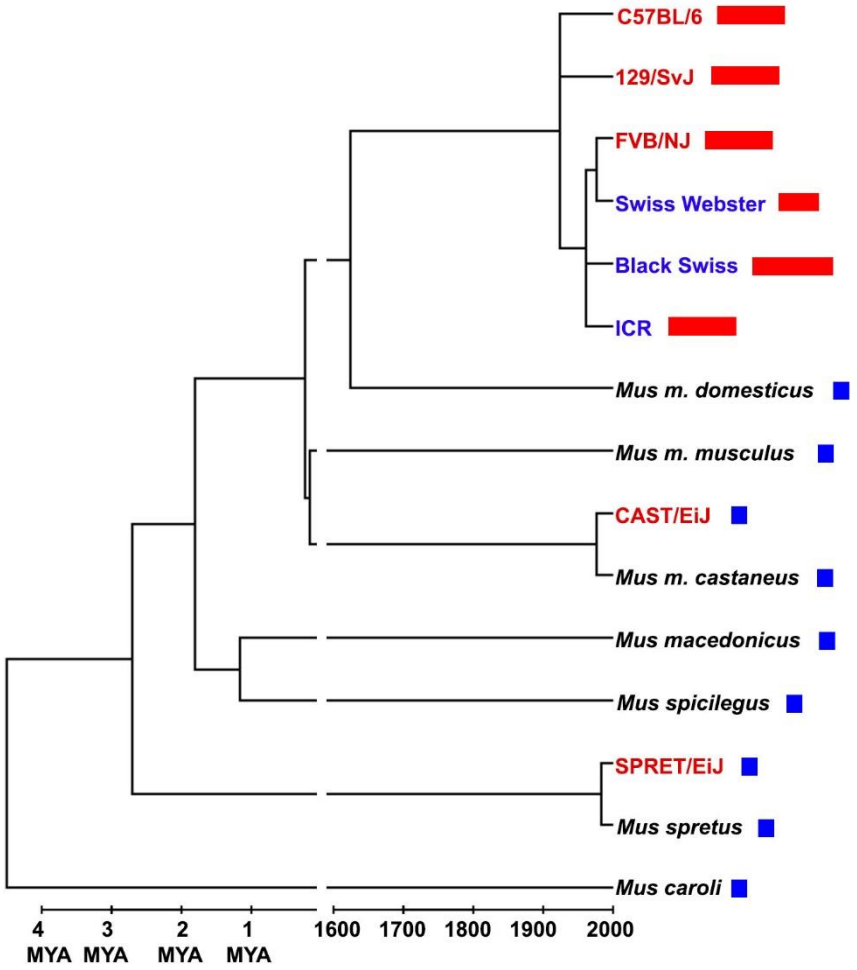

Figure 1. Telomere length in mice. Text in red denotes inbred strains, blue text is for outbred strains and black text is for wild-trapped individuals. Bars represent telomere length (long telomeres in red, short telomeres in blue). Timeline is broken in order to show millions of years ago (MYA) on the left and the last four centuries on the right. Data and references are in **Table S1** in Supplementary material.

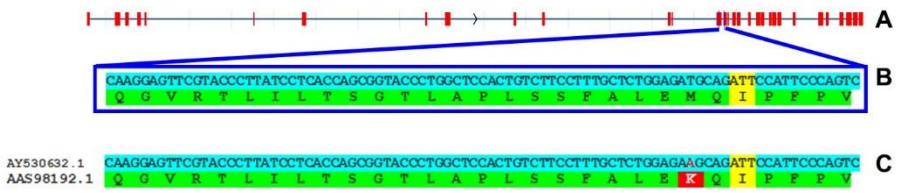

Figure 2. RTEL1 protein sequence in *Mus spretus*. A Gene structure (exons in red). **B** Zoom of exons 17-18 (nucleotides from genome assembly SPRET_EiJ_v3 in cyan, newly predicted amino acids in green, splice site in yellow). **C** Same as in **B**, but using GenBank-deposited sequences (red denotes nucleotide variation and consequent change in 492 from M to K).

Because of a false premise (i.e., K492 in *Mus spretus*), the Telomouse does not mimic the condition in the Algerian mouse. Nonetheless, it is a useful inbred strain (congenic with the popular C57BL/6) with human-like telomere length. Moreover, it could recapitulate the RTEL1-related Hoyeraal-Hreidarsson syndrome (HHS), since many patients with this disease show the M492I variation[3]. Someone could object that Telomouse is a bad model for recapitulating HHS, because mice have telomerase activity and humans have not. However, this is an over-simplification (although very

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popular): in reality, many human cells (especially, but not limited to, hematopoietic stem cells) show telomerase activity[4].

Beside the potential use of Telomouse, the results obtained by Smoom et al. open up new intriguing questions. How much the telomeres of Telomouse fibroblasts would shorten in vitro if grown at physiological oxygen levels (i.e., 5% instead of the usually employed atmospheric 20%). Is the telomere shortening observed in vitro and between generations of Telomouse ascribable only to a decrease in telomerase activity at telomeres? Or the reduced activity of RTEL1 has consequences also on Alternative Lengthening of Telomeres (ALT)? The authors found no differences in telomeric recombination (indicative of ALT) in vitro, but this does not exclude differences, for example, in oocytes and cleavage stage embryos. In these, in fact, telomere elongation has been observed also in telomerase-null mice and ALT activity has been reported[5].

Another important question, not strictly related to Telomuse, is the link between RTEL1 and the presence of very long telomeres in laboratory mice. Smoom et al. already indicated that M492 is present in rodent species (excluding *Mus spretus*, according to them) with short telomeres and thus cannot explain telomere length differences between these and laboratory mice[1]. Here I demonstrated that this is valid also for *Mus spretus*. Moreover, I found that there is no amino acid that is conserved in short-telomeres *Mus* species and is modified in long-telomeres strains (Figure S2 in Supplementary material). Therefore, differences in telomere length cannot be ascribed to differences in RTEL1 protein sequence. Nonetheless, the *Rtel1* gene was at first discovered as a chromosome locus responsible for telomere length differences between *Mus spretus* and BALB/c[6] mice and a subsequent study showed that in crosses between *Rtel1*$^{+/-}$ laboratory mice and *Mus spretus*, the ones with *Rtel1*$^{+}$ allele had long telomeres and the ones with *Rtel1*$^{-}$ had short telomeres[2]. Different explanations can be proposed: 1) the study of Ding et al.[2] had some unidentified error and RTEL1 is not responsible at all for differences in telomere length; 2) differences between *Mus* species/strains are due to differences in the relative abundance of RTEL1 isoforms; 3) differences in telomere length are due to the *Rtel1* gene, but the variations are in the promoter (causing different levels of transcription) and not in the coding sequence. I found that among the conserved (in wild species) nucleotides in the promoter, only two are modified in laboratory strains: one of the two is a C>T modification, changing a -CAAC- into a -CAAT-. However, although attractive, it does not really resemble a CCAAT box and seems to far from the transcription start site. In any case, these hypotheses can be easily tested by quantitative PCR and Western blot, in order to assess relative abundance of variant transcripts and protein isoforms.

The last issue (which in my personal opinion is the most important) regards telomere length in *Mus musculus*. The mystery of how and why long telomeres appeared in laboratory strains has not been solved: it seems like it is not due to inbreeding (Figure 1) and many hypotheses can be raised on domestication of mice and increase of mating period, ALT and telomerase activity in germ cells, epigenetics and so on. But beside these questions, one thing should be clarified: *Mus musculus* (and even *Mus musculus domesticus*) has NOT long telomeres. This is a characteristic of laboratory strains (for the history of their ancestry, see[7]) and not of the species (or subspecies). Not grasping this aspect can lead to errors and false conclusions in studies on inter-species comparison or when physiological and life history traits are regressed against telomere length. For example, because of the plausible link between telomeres and aging, many researchers have regressed telomere length[8-9] (or telomere shortening rate[10-11]) against maximum lifespan. However, the telomere length used for *Mus musculus* is always the one of laboratory strains (40-100 kbp, heavily influencing every regression) and the maximum lifespan is the one of wild mice (4 years[12]). Similar considerations can be made on studies on the evolution of telomeres in mammals[9]. In conclusion, when dealing with telomere length, attention should be paid to the differences between *Mus musculus* as a species and laboratory strains.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Data Availability: All data generated or analysed during this study are included in the published article and in the Supplementary material. RTEL1 sequences have been deposited in GenBank with accession number XXXXX.

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Competing Interests: The author declares no competing interests.

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