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Expression level of R155H mRNA in the knock-in mouse model

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Dear Editor,

We read the article by Clemen et al. entitled as "The heterozygous R155C VCP mutation: Toxic in humans! Harmless in mice?" [1] with great interest. In this article, authors aimed to establish IBMPFD model by generating a mouse harboring a common patient-specific mutation R155C. After a battery of behavioral, histological and biochemical analyses, they found that R155C heterozygous mice did not manifest the profound pathology found in human IBMPFD patients. To understand why R155C VCP knock-in led to no phenotypic changes, Clemen et al. analyzed the amount of wildtype and mutant VCP mRNA species in the R155C heterozygous mice, and indicated that there was only 5% and 7% mutant mRNA detected in the skeletal muscle and the brain tissue, respectively. This result suggests that in the R155C heterozygous mice, the wildtype VCP mRNA dominates, and thus produce functional VCP protein.

It is intriguing to see the unequal distribution of wildtype and mutant VCP mRNA species in the R155C heterozygous mice. Previously published results in IBMPFD mouse model harboring patient-specific mutation R155H have suggested that heterozygous R155H mice display age-dependent muscle weakness. Furthermore, histology and biochemical analyses suggest that R155H heterozygous mice form centralized muscle nuclei, upregulation of TDP43 and inclusion bodies positive for TDP43, VCP and Ubiquitin, recapitulating the phenotypes manifested in human patients [2]. Therefore, we investigated whether the mRNA findings in the R155C mice was also observed in the R155H heterozygous mice. The mRNA from heart and skeletal muscle of R155H heterozygous, R155H homozygous and wildtype control mice were analyzed to estimate the amounts of wildtype and R155H mRNA. Equal amount of PCR products were subjected to NciI restriction enzyme digestion, where the presence of R155H mutation destroys the endogenous NciI site. As expected, the VCP mRNA from wildtype mice were completely digested into two smaller fragments. However, VCP mRNA species from R155H heterozygous mice were largely intact upon NciI cutting, similar to what was observed in R155H homozygous mice. Also, a partial digestion (three bands) was observed in NciI cutting of PCR product containing equal mixture of wildtype and homozygous cDNA (Fig. 1). These results indicate that the VCP mRNA from R155H heterozygous mice may contain a large portion of mutant VCP, giving rise to the mutant VCP protein and further physiological abnormality in mice. It is possible that different VCP mutations can impact mRNA synthesis or metabolism differently. In the case R155C, a decrease of VCP mutant mRNA expression (or increase in degradation) is presumed to play a protective role in the R155C heterozygous mice.

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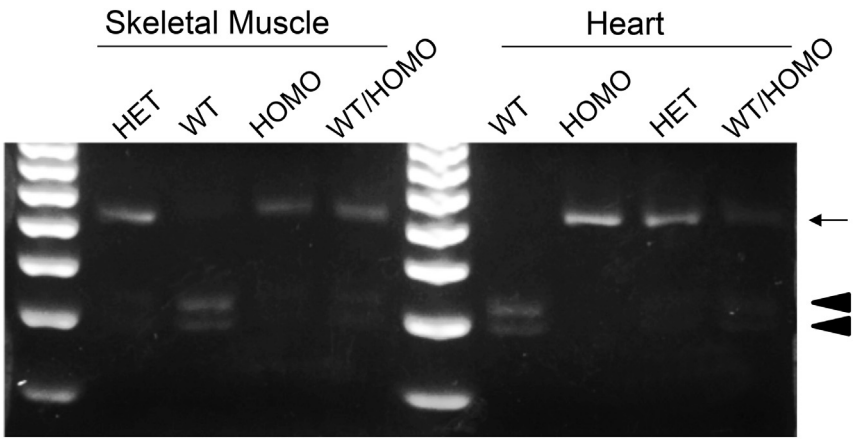

Fig. 1. Expression level of wildtype and mutant R155H VCP mRNA in skeletal muscle and heart of heterozygous (HET), homozygous (HOMO) and wildtype (WT) mice. mRNA from three genotypes were extracted, reverse transcribed and PCR amplified to obtain fragments containing VCP R155H mutation. The PCR products were then subjected to NciI restriction enzyme digestion. WT/HOMO represents the digestion results from PCR amplified with 1:1 mixture of WT and homozygous cDNA. Arrow: the full length PCR product. Arrowhead: the digested products.

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