## Additional Discussion for Implicated Genes

The region on chromosome 7 is closest to the gene *Catsperg2*, which is a calcium ion channel associated with sperm activity (Wang *et al.* 2009). Although this is yet another calcium homeostasis gene and provides for some interesting hypotheses, unpublished data demonstrates no clear differences between HR and C sperm (S. A. Kelly and T. Garland, Jr., personal communication). Additionally, the more differentiated loci within this region are downstream of this gene and cover an expanse with no verified annotations.

A chromosome 11 region including *Sox9* was found to be differentiated. Sox9 is a transcription factor with extensive influence on a vast variety of phenotypes. Many of these are associated with sex determination (Jakob and Lovell-Badge 2011) and cartilage development (Lefebvre *et al.* 2019), the latter having large implications for skeletal development (Bi *et al.* 2001). Knockouts have also generated changes in heart (Lincoln *et al.* 2007) and neural (Cheung *et al.* 2005) development. With the widespread implications this gene could have for the HR mice, more research will have to be done to understand the specific role of *Sox9* in voluntary wheel-running behavior.

On chromosome 16, *Tnk2* (aka *ACK1*) has elevated expression levels in the brain as compared with other organs, with further expression increases during development (La Torre *et al.* 2006). *Ubxn7* knockouts have produced mice with stunted growth (The International Mouse Phenotyping Consortium *et al.* 2016). But the seemingly most relevant gene for the HR and C differentiation is *Fbxo45*. *Fbxo45* is essential for neuronal development (Saiga *et al.* 2009) and synaptic transmission (Tada *et al.* 2010). Furthermore, this gene is shown to interact with N-cadherin (Cdh2, neuronal cell adhesion molecule important for neuronal development) in a calcium-dependent manner to stabilize N-cadherin (Chung *et al.* 2014).

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