Holly Heacock
Specific Aims

Prader-Willi Syndrome (PWS) is a multisystem genetic disorder caused by a deletion on the paternal chromosome 15 resulting in the loss of five genes' expression[(1)]. One of these genes is necdin (NDN) which primarily plays a role in neural tissue differentiation[(2)]. NDN is found only in mammals and thus, mice have been the main model organism used in studies. However, mouse NDN is expressed solely in the brain in comparison to the ubiquitous expression found in human NDN leading to gaps in how NDN expression affects other areas of the human body[(3)]. Rhesus macaques have recently been used as model organisms to study PWS mechanisms because they have a closer common ancestor to humans than mice[(4)]. It is possible that the rhesus macaque could be a better model organism to study NDN expression and what NDN deletion causes. However*, it is unknown whether or not humans have a more similar expression pattern to the rhesus macaque than to mice, making the rhesus macaque a better organism of study.*

My **primary goal** is to discern the differences and similarities between the human, mouse, and rhesus macaque NDN expression with hopes of the rhesus macaque being a more informative study organism for PWS. My **hypothesis** is that rhesus macaque NDN will show more similar sequences and expression patterns to humans than to mice because of its closer evolutionary split and sequence homology. In studying this, my **long term goal** is to use rhesus macaques as a better model organism in determining how human NDN deletion creates specific symptoms of PWS in humans in order to create better drugs and/or treatment options.

Aim 1: Determine sequence similarities and differences of human, mice, and rhesus macaque NDN to assess if rhesus macaques have a closer similarity in sequence to humans than mice.
Approach: Use BLAST to align and determine sequence similarities and differences and SMART/Pfam to ascertain protein domain similarities and differences across the three species.
Hypothesis: Humans will have a higher percentage alignment and more similar domain locations to rhesus macaques than to mice.

Aim 2: Determine NDN gene expression changes in different tissues of the rhesus macaque compared to humans and mice.
Approach: Use RNA-Seq transcriptomic analysis to uncover gene expression levels in different tissues at the RNA level and compare to immunohistochemistry samples of those same tissues.
Hypothesis: Humans will have more similar expression levels of necdin RNA to rhesus macaques than to mice.

Aim 3: Determine NDN protein expression tissue locations in rhesus macaques compared to mice and humans
Approach: Use mass spectrometry to establish presence and relative abundance of necdin protein in different tissues within each organism and compare to data on UniProt databases.
Hypothesis: Rhesus macaques will have necdin protein within all tissues of the body while mice will only have expression in brain tissue.

Studies on rhesus macaques have been increasing in terms of PWS gene mechanisms[(4)]. I hope that by using a new model organism more similar to humans to study the effects of the loss of NDN, we may be able to determine further areas of study for PWS defects.

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References

1) Jay, P., Rougeulle, C., Massacrier, A., Moncla, A., Mattel, M., Malzac, P., ... Muscatelli, F. (1997). The human necdin gene, NDN, is maternally imprinted and located in the Prader-Willi syndrome chromosomal region. *Nature Genetics, 17*, 357-361.

2) Macdonald, H., & Wevrick, R. (1997). The Necdin Gene is Deleted in Prader-Willi Syndrome and is Imprinted in Human and Mouse. *Human Molecular Genetics,6*(11), 1873-1878.

3) NDN - Necdin - Homo sapiens (Human). (n.d.). Retrieved February 26, 2015, from http://www.uniprot.org/uniprot/Q99608

4) Fujimoto, Akihisa, Shoukhrat M. Mitalipov, Hung-Chih Kuo, and Don P. Wolf. "Aberrant Genomic Imprinting in Rhesus Monkey Embryonic Stem Cells." *Stem Cells* 24.3 (2005): 595-603. Print.