**Discovery of possible connection between cln3 gene and cisd2 gene through notch signaling.**

**I. Introduction**

CISD2 is a gene found in humans that is known to cause Wolfram syndrome, a neurodegenerative disease.1 Wolfram syndrome is a genetic disease passed through autosomal chromosomes, or any chromosome that is not the X or Y sex chromosomes.1 Wolfram syndrome is associated with diabetes insipidis, a condition in which the body has difficulty processing fluids as a result of a hormone abnormality.1, 2 This results in unbalanced salt and water metabolism. Wolfram syndrome also causes diabetes, damage to optic nerves eventually leading to blindness and deafness as well as psychiatric illness and renal-tract abnormalities.1, 2

CISD2 recessive mutations are believed to be a cause Wolfram Syndrome 2.1,3 CISD2 produces proteins that are found around the ER and the mutation of CISD2 causes Ca2+ imbalance, this imbalance caused an increased amount of ER and mitochondria contact.3 This causes dysfunction in the mitochondria, a possible cause of some of the diseases and problems associated with Wolfram syndrome 2.1,3 The complete function of CISD2 is still uncomplete however, there is still much to learn about this gene and its mutations.1,3

CLN3 is another gene with neurological problems associated with it; CLN3 is the gene whose mutation causes Batten disease.4 Batten disease is a neurodegenerative disease that begins in children around 5-7 years old with retinal degeneration.4 It leads to seizures, loss of motor skills and mental ability; death normally occurs by 25 years of age.4 CLN3 gene encodes for a multi-spanning, hydrophobic transmembrane protein. Though the function of this particular protein is unknown, it is now known that CLN3 interacts with the notch pathway.4 The notch pathway is a signaling pathway, a cell-to-cell contact dependent signaling mechanism used for cells to communicate with each other. This particular pathway is believed to be important in development and therefore mutations in this receptor lead to abnormalities in development.5,6,7

Because CLN3 and CISD2 both are genes whose mutations lead to neurogenerative disorders, it would be greatly useful to study these genes, however, because studying genes in humans leads to obvious complications another way to study these genes would be necessary. Flies and humans have amazingly had similar genes both for CISD2 and CLN3. CG1458 in flies was identified as the best match for CISD2 gene in humans.1 From here on CG1458 shall be cisd2 and CLN3 in humans shall be cln3 for flies.1

Jones et al (2014) found a connection between cln3 and cisd2. They found that a particular phenotype that appeared in flies that had a cln3 over-expression was worsened in flies that also had a cisd2 knockdown.1 Gene knockdown is a way to silence genes or prevent them from being expressed. They had blocked cisd2 from being expressed in flies with a cln3. Black spots on the eyes of the flies caused by the cln3 overexpression were worsened, increased in size and amount, with the cisd2 knockdown. These results indicate that these two genes interact with each other. They speculated that one possibility is that cln3 mediated notch signaling may be important for the function of some of these genes, including cisd2. Ideally, cln3 and cisd2 genes and their function are somehow molecularly connected to each other, through this pathway. So, if one gene is functioning improperly it would cause the entire system to become dysfunctional. The experiment in this proposal is to test if this possibility is true.

**II. Experiment**

This experiment is going to determine whether the knockdown of the cln3 meditated notch signaling in flies with cln3 overexpression causes a particular phenotype to appear and compare that to flies with cln3 overexpression alone. Eventually, I also hope to test flies with cln3 mediated notch signaling knockdown and flies with cisd2 knockdown and compare that to the previous results (no cisd2 knockdown). If these two genes are connected via the notch signaling pathway I would expect that the phenotype seen with cln3 overexpression to worsen in severity or become severe; similarly, I would expect the phenotype to become more severe if cln3 were overexpressed and cisd2 were knockdown if these two are connected through this pathway.

Gal4-UAS system (gene knockdown)

In order to knockdown the genes, to manipulate its expression, I would use the Gal4-UAS system also used in experiments by Jones et al (2014).1 The Gal4-UAS system allows for targeted gene expression, selective activation of any cloned gene.9 The Gal4-UAS system also allows the selective activation to be tissue or cell-specific patterns.9 GAL4 a transcriptional activator in yeast can be used as a transcriptional activator in flies if the promoters have GAL4 binding sites. The genes need to be responsive to the GAL4 genes so a vector of five GAL4 binding sites lined up was designed. Genes could then be subcloned on this vector. This is known as the Upstream Activation Sequence or the UAS.9 Using this system I can subclone any sequence behind a GAL4 binding site, activate the target gene only in cells where GAL4 is expressed and this system allows for observation of the effect on development (it affects phenotype). 9

Jones el al (2014) also used the Gal4-UAS system to manipulate cisd2 expression in their experiments to find if there was a connection between the two genes expression or lack of expression. They used the Gal4-UAS system to make two RNA interference transgenes that would manipulate cisd2 expression.1 They created two transgenes that through immunoblot and qRT-PCR tests were shown to have a loss of function for cisd2.1 The same system would be used to knockdown the cln3-manipulated signaling and to knockdown cisd2 in later experiments.

RNA interference transgenes (RNAi transgenes) are strand of RNA created from either the cell or researcher inserting a specific dsRNA strand into the cytoplasm of the cell. This then is processed through the RNA-induced silencing complex (RISC). The dsRNA is processed and guides the RISC to where the complementary RNA is. RISC can then silence the targeted genes.

The specific knockdowns will be created using the Gal4-UAS system and then inserted into the cell cytoplasm. Once in the cytoplasm the RISC will take it and create RNAi transgenes that will block that specific function of the gene. A specific phenotype will result from the transgenes used; depending on whether both cisd2 and cln3 mediated notch signaling are knockdown, whether one is knocked down, or both are knocked down

The phenotype will be the measurement for this experiment, the particular phenotype that will result is that the round optical pieces that make up the eye of a fly will be black instead of the normal color, kind of reddish. A similar scale to the one used in Jones et al. (2014) experiment will be used; None – no black spots, Mild – a few black spots, Medium – one or more patches of black spots, severe – black spots throughout the eye.

**III. Discussion**

The best outcome for this experiment is a clear difference between the different trials of this experiment. If the outcomes of the different trials (both cisd2 and cln3 mediated notch signaling are knockdown, whether one is knocked down, or both are knocked down) all end with the same or too similar of a result the idea of them being linked will be questionable – though considering the experiments from Jones et al (2014) this outcome is unlikely. The most important difference that needs to be present is a difference between when the notch signaling is knocked down and when it isn’t knocked down.

The ideal results would be if the phenotype, when the cln3 mediated notch signaling is not present, or knocked down, was worse or severe. Ideally, both with cisd2 and without cisd2 knockdown. These results would show that cisd2 and cln3 are connected via this notch signaling and that the hypothesis was correct. However, if the phenotype is less or mild, perhaps even none appears, then that would show the opposite, that the hypothesis is wrong, they are not connected via notch signaling. If this happens then it’s possible they are just connected via a different kind of signaling like JNK.4

I would really rather find a way to measure this interaction molecularly, perhaps find the particular part of notch signaling or find the specifics of how these two communicate via this pathway (assuming of course this is the pathway they communicate through). However, I don’t know of any way to do this.

The biggest problem with this experiment is that the knockdown of the genes somehow causes a reaction that messes up the phenotype I am looking for, this is unlikely due to the precision of the Gal4-UAS system but it is a possibility. The biggest setback for this experiment would be if the results are inconclusive. Then that means that notch signaling isn’t the connection but there are multiple other routes that would need investigating then if that is the case.

Regardless of these problems knowing how these genes function, together and separately, is key in our understanding of genetics. It may help shed light on how other genes work together, helping us to understand genetic diseases and our own genetic code just a little bit more. Perhaps in the future genetic disease and resulting deaths and suffering will be a thing of the past.

**IV. References**

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