

THE PATHOPHYSIOLOGY OF AUTOSOMAL DOMINANT ADULT-  
ONSET NEURONAL CEROID LIPOFUSCINOSIS AND THE ROLE  
OF CYSTEINE-STRING PROTEIN ALPHA

By

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A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors Degree  
With Honors in

Physiology

THE UNIVERSITY OF ARIZONA

SPRING 2017

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## **I. Abstract**

This thesis serves as a review of the current literature and knowledge surrounding autosomal dominant Adult-Onset Neuronal Ceroid Lipofuscinosis, its underlying mutations, and disease pathophysiology. ANCL has been extensively researched in the past two decades and the major breakthroughs regarding this neurodegenerative disease are highlighted in the following pages.

The mutations L115R and L116 $\Delta$  in the DNAJC5 gene encoding cysteine-string protein alpha have been identified as the underlying source of ANCL. Impaired functioning of CSP $\alpha$  somehow leads to neurodegeneration, however its pathogenic pathways remain unclear. Recognizing the mechanisms by which mutant CSP $\alpha$  may lead to disease-onset is the key to understanding ANCL and, therefore, the focus of this literature review.

First, a general introduction to various physiological pathways essential for understanding ANCL is provided. This sets the stage for part II, in which the current literature on NCL is consolidated, allowing the pieces of the ANCL puzzle to fall together. This synopsis is given as persuasion for an overarching process of ANCL disease pathology involving many complex mechanistic pathways combined.

## II. Introduction

The etiology and pathogenesis of neurodegenerative diseases is a complex field of study to say the least. Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis are only a few of the neurodegenerative disorders afflicting the human race. Maintaining cognitive function throughout the span of a lifetime can be a nearly impossible task, as genetic polymorphisms can more or less seal one's fate.

Neuronal Ceroid Lipofuscinosis (NCL) is a class of inherited neurodegenerative diseases with no known cure (Benitez 2011). A rare form of this disease, known as autosomal dominant Adult-Onset NCL, will be the topic of this literature review. Although many important insights have been made since the discovery of ANCL, the mechanisms leading to neurodegeneration are still the focus of intense research. Recognizing these mechanistic pathways is imperative in understanding the disease and eventually developing medications that may reverse or inhibit ANCL.

ANCL is caused by dominant mutations in the DNAJC5 gene encoding cysteine-string protein alpha ( $CSP\alpha$ ), a chaperone protein involved in many processes essential for synaptic maintenance and neurotransmission (Zhao et al. 2008; Zinsmaier and Bronk 2001; Zinsmaier 2010). Two dominantly inherited mutations in  $CSP\alpha$ , L115R and L116 $\Delta$ , seem to be linked to the impaired neurotransmitter release seen in ANCL (Benitez et al. 2011; Greaves et al. 2012; Velinov et al. 2012; Cadieux-Dion et al. 2013). The pathways by which synaptic transmission is inhibited, however, remain unclear. Since  $CSP\alpha$ 's discovery less than three decades ago, research has provided many valuable insights regarding ANCL pathology.

## **Neurotransmission**

### *Overview of Synaptic Transmission*

Neuronal signaling is a complicated process, involving many components working together. In order for any living being to perform voluntary or involuntary actions, register sensations, and process information, nervous tissue must be capable of intercellular communication. A neuron consists of a soma, or cell body, from which appendages called axons and dendrites project outward. The axon can range in length from several millimeters to macroscopic length and ends in axon terminals, also known as synaptic boutons, in which vesicles containing neurotransmitters are held. The synapse refers to a presynaptic neuron's axon terminals, a synaptic cleft, and a postsynaptic neuron's dendrites. Neurotransmitters are released from the presynaptic axon terminals into the synaptic cleft and bind to receptors on the postsynaptic neuron's dendrites. Depending on the type of receptor and neurotransmitter, this binding can either open or close membrane ion channels. As positive charge flows in through the ion channels, the neuron becomes increasingly depolarized, until a threshold is reached and integrated into an action potential at the axon hillock. An action potential is an electrical impulse, which travels down the axon in order to transmit signals from one neuron to the next. Once the action potential reaches the axon terminal, voltage-gated calcium channels change their conformation and allow  $\text{Ca}^{2+}$  to flow through a channel pore, triggering the exocytosis of synaptic vesicles and their contents (Katz and Miledi 1969). Each vesicle contains about 7,000 neurotransmitters, permitting rapid synaptic transmission to the postsynaptic neuron.

The synaptic vesicle protein components are then recycled to form new synaptic vesicles, permitting rapid and sustained neurotransmitter release during extended periods of neuronal activation (Zinsmaier and Imad 2011). With such a highly advanced system of exocytosis and endocytosis, molecular chaperones are necessary to assist in the process. Chaperones are involved in a large variety of neuronal activities, some of which include assisting in neuronal signaling by regulating protein activities involved in synaptic activities; facilitating protein folding; and playing critical roles in synapse maintenance (Zinsmaier and Bronk 2001; Zhao et al. 2008; Zhang et al. 2014). Without these chaperones, misfolded and dysfunctional proteins accumulate, eventually leading to neurodegenerative diseases (Goldstein 2003; Levine et al. 2004; Zhang 2014).

### *SNARE-Complex Formation*

Fusion of neurotransmitter-containing vesicles and signaling between neurons requires the formation of a SNARE-complex linking the vesicular membrane and the cellular membrane (Soellner et al. 1993). SNARE stands for soluble NSF (N-ethylmaleimide sensitive factor) attachment receptors. The main components of this SNARE complex are the Q-SNAREs, syntaxin and SNAP-25, embedded in the cellular membrane and the R-SNAREs of the VAMP (vesicle-associated membrane proteins) gene family, synaptotagmin and synaptobrevin, embedded in the vesicular membrane. When an action potential reaches the nerve terminal, the depolarization causes voltage-gated  $Ca^{2+}$  channels to open, allowing calcium to flow in. Calcium ion influx initiates the fusion of synaptic vesicles with the plasma membrane, permitting the vesicles to export their contents out of the cell.

## **The Lysosome**

### *Lysosomal Trafficking*

In order to maintain homeostasis, cells continuously synthesize, break down, and recycle biomolecules necessary to sustain life. Thus, mammalian cells contain lysosomes, membrane-enclosed organelles whose primary function is to break down intracellular wastes by way of their digestive enzymes. The pH of the lysosome is low, at around 4.5-5.0, when compared to the surrounding cellular environment with a relatively neutral pH of about 7.2. This acidic pH provides the optimal condition for lysosomal enzymes to digest cellular contents. The lysosome is able to maintain its acidic environment because of proton pumps found throughout its plasma membrane. This type of proton pump is known as a vacuolar H<sup>+</sup>-ATPase because it uses energy from ATP hydrolysis to pump hydrogen ions against their concentration gradient into the lysosome.

Lysosomal proteins can reach the lysosome directly, through the trans-Golgi network or indirectly, from the plasma membrane (Schultz et al. 2011). If an enzyme is tagged with mannose-6-phosphate (M6P) while it is trafficked to the trans-Golgi network, it will be recognized by M6P receptors and consequently targeted to the lysosome.

Digestive enzymes of the lysosome are formed and folded in the rough endoplasmic reticulum, packaged and labeled in the Golgi, then sent out through the trans face of the Golgi in vesicles. Meanwhile endosomes become increasingly acidic because of vacuolar H<sup>+</sup>-ATPase pumps studding their membranes. As the early endosome becomes more acidic and other factors drive its maturation, it eventually matures into a late endosome. The late endosome fuses with the vesicle from the Golgi to provide the cell with the lysosome.

The lysosome can receive its substrates through various pathways such as endocytosis, phagocytosis, and autophagy (Schultz 2011). When extracellular components are taken into the cell, this is referred to as endocytosis. The membrane of the endocytosed vesicle is studded with proteins to be degraded and recycled. Thus, the lysosome can break down both intracellular and extracellular materials. Phagocytosis is the mechanism of substrate intake in which the lysosome's plasma membrane engulfs the intracellular molecule or object to be degraded, forming a phagocytic vesicle inside the lysosome. Autophagy is mediated by autophagosomes, which are vesicles that engulf a portion of the cytoplasm containing molecules to be degraded. The autophagosome then delivers unnecessary and/or dysfunctional biomolecules to the lysosome.

The lysosomal membrane has many important cellular functions including mediation of fusion with other vesicles, separation of its acidic pH from the surrounding environment, and facilitation of lysosomal content exocytosis. Its membrane contains many proteins imperative for fusion with other endosomes and vesicles. However, the specific functions of many of these proteins are still unknown. Certain SNARE proteins similar to those of vesicular fusion allow the lysosome to bind with other endosomes to bring in cellular wastes. The lysosomal membrane also contains transporters allowing the lysosome to export its hydrolyzed substrates (i.e. individual amino acids, sugars, lipids, etc.) back into the cell to be reused.

The loss of lysosomal functioning has catastrophic implications and is thought to be the underlying cause of certain neurodegenerative disorders, such as neuronal ceroid lipofuscinosis. If there were no lysosomes, all sorts of excess and dysfunctional biomolecules, macromolecules, and cellular debris would rapidly accumulate in the

cytosol. It has been theorized that this accumulation, commonly seen in neurodegenerative diseases, leads to a pathologic disruption in normal cellular activity.

### *Ubiquitination Targets Membrane-Associated Proteins to the Lysosome*

In order for a membrane-associated protein to be marked for degradation by the lysosome, it must be ubiquitinated. Ubiquitination is a cellular mechanism for regulating the location and recycling of all proteins. Furthermore, ubiquitination can facilitate interactions between proteins, thus playing an essential role in normal neuronal development and function (Franco 2010). If this pathway fails, neurodegeneration can result, as proteins that would normally be targeted to the lysosome remain inside the cell. Eventually these proteins will accumulate and the result can be devastating.

Proteins are ubiquitinated by means of a three-enzyme ubiquitination pathway. The first enzyme, E1, activates ubiquitin. Through ATP hydrolysis the carboxyl end of ubiquitin is linked to the E1 enzyme. The second enzyme in this pathway, E2, is known as the conjugating enzyme. Activated ubiquitin is transferred onto the second enzyme, where it is attached to one of E2's cysteine residues. The third enzyme in this pathway, E3, is a ligase, which transfers ubiquitin from E2 to a lysine residue on the substrate to be ubiquitinated. If a substrate becomes polyubiquitinated, it is typically targeted to a proteasome. Attachment of only one or a few ubiquitin monomers will normally result in endocytosis of the substrate into a lysosome (Chau et al. 1989). In either case, ubiquitination is generally associated with targeting cellular substrates for degradation in the cell and as such, is a very important mechanism in the regular functions of the lysosome.

### **III. Literature Review**

#### **Neuronal Ceroid Lipofuscinoses**

##### *Overview of NCL*

Neuronal ceroid lipofuscinosis (NCL), also known as Batten Disease, is a class of neurodegenerative disorders characterized by the accumulation of lysosomal autofluorescent ceroid-lipofuscin aggregates in neuronal tissues (Hobert and Dawson 2006; Mole et al. 2010). These aggregates are a “complex mixture of proteins, lipids, and metals”, which can be identified due to their fluorescent nature (Benitez 2015). Thus far, understanding the exact constituents and origins of these accumulations does not seem to yield a better understanding of the mechanisms underlying the NCL disease class.

Research suggests that the lipofuscin aggregates can be present without disease progression suggesting that these aggregates are correlated but not causative of the disease. The physiological manifestations of NCL include seizures, gradual cognitive and motor decline, visual impairment, and premature death (Warrier et al. 2013). The neurodegeneration in neuronal ceroid lipofuscinosis is widespread, affecting cerebral, cerebellar, and retinal nervous tissues.

NCL is classified as a genetically heterogeneous disease class; meaning mutations in different genes can cause the same type of disease. They are also clinically heterogeneous, meaning every form of this disease displays variable severity, onset, and/or symptoms. The classification of the neuronal ceroid lipofuscinoses relies heavily on this heterogeneity, and a nomenclature scheme has been carefully formulated to give a better understanding of the different NCL disorders (Williams 2012). The most up to date nomenclature of NCL is an axial diagnostic system consisting of the following seven

axes: the affected gene, mutation diagnosis, biochemical phenotype, clinical phenotype, ultrastructural features, functionality, and any further remarks.

### *NCL Genes and Their Related Proteins*

Neuronal ceroid lipofuscinosis is classified as a lysosomal storage disease, manifesting in many different forms depending on the gene of mutation. The mutated products of the different forms of NCL range from soluble proteins (i.e. lysosomal enzymes) to lysosomal and endoplasmic reticulum membrane proteins and other proteins involved in secretion and/or found in the cytosol (Bennett 2013; Simonati et al. 2014). Thus far, fifteen CLN genes have been identified through genome sequencing, which are linked to NCL in its multitude of mutated forms (Carcel-Trullols et al. 2015).

The most common forms of NCL are autosomal recessive and display onset in the infantile or juvenile years. In fact, neuronal ceroid lipofuscinosis is considered the most common group of childhood inherited neurodegenerative disorders. Among the infantile neuronal ceroid lipofuscinoses (INCL), the CLN1/PPT gene mutation seems to be the most prevalent and most severe form of NCL.

PPT (also known as CLN1) is a gene, which encodes for palmitoyl protein thioesterase 1 (PPT1), a soluble lysosomal enzyme (Vesa et al. 1995; Cotman 2013; Simonati et al. 2014). More specifically, this protein “cleaves the fatty acids from S-palmitoylated peptides” in the lysosome during degradation (Hellsten et al. 1996; Bennett et al. 2013). Examples of S-palmitoylated peptides include those involved in endocytosis and exocytosis, such as dynamin, SNAP-25, synaptotagmin, CSP, and synaptobrevin 2 (Gundersen et al. 1994). A loss of function in PPT1 leads to an accumulation of

palmitoylated synaptic proteins and although the mechanisms producing disease are not yet fully understood, it is possible that this accumulation of proteins in the presynapse leads to neurodegeneration (Aby et al. 2013).

The onset of CLN1/PPT occurs within the first 2 years of life and is characterized by developmental impairment, retinal degeneration, seizure disorders, sleep disturbance, and mobility issues. Following this rapid neurodegeneration, children typically enter a vegetative state until death in the early teenage years. Other clinical findings of this disease include a complete absence of (or dysfunctional) PPT1; aggregations of saposins A and D (sphingolipid activator proteins), which comprise the majority of the autofluorescent lipofuscin accumulation; and granular osmophilic deposits (Mole et al. 2005).

Other genes encoding soluble lysosomal proteins are CLN2/TPP1, CLN5, CLN10/CTSD, and CLN13/CTSF. Many of these genes encode enzymes that target specific substrates and residues to be cleaved in the lysosome, similar to PPT1 (Simonati et al. 2014; Carcel-Trullols et al. 2015). Other genes causative of NCL in their mutated forms encode lysosomal membrane proteins (CLN3, CLN 7, and CLN12) as well as proteins localized to the endoplasmic reticulum and Golgi membranes (CLN6 and CLN8). Another more recently identified gene is CLN14, with homology to a component of the  $K^+$ -channel thought to facilitate hyperpolarization of the neuronal cell membrane (Carcel-Trullols et al. 2015). Since NCL are classified as lysosomal storage diseases, there has been some debate as to whether the NCL-linked genes encoding proteins unrelated to the lysosome can be branded as NCL's. However, the lack of knowledge surrounding our current understanding of NCL's makes it quite possible that these genes

may facilitate lysosomal degradation in some unknown way. In addition to cytosolic and membrane proteins, mutated genes of NCL can also yield secretory and cytosolic proteins, such as CLN4.

There are three types of NCL expressing onset in adulthood. The focus of this thesis is the rare form of NCL caused by mutations in CLN4/DNAJC5. This gene encodes cysteine-string protein alpha, present on synaptic vesicle membranes among other locations throughout the body (Simonati et al. 2014). This is the only form of NCL presenting in adulthood with autosomal dominant inheritance, making up only a mere 10% of NCL cases (Sadzot et al. 2000). This subset of NCL is called autosomal dominant Adult-Onset Neuronal Ceroid Lipofuscinosis (AD-ANCL), also known as Kufs disease. Another adult-onset form of NCL not extensively outlined in this thesis is a recessively inherited form of Kufs Disease associated with mutations in CLN6 (Arsov et al. 2011).

#### *AD-ANCL*

Autosomal dominant adult-onset neuronal ceroid lipofuscinosis is the term describing the rare form of NCL's that present in adulthood. This subset of neuronal ceroid lipofuscinosis has many of the same characteristics as its childhood-onset counterparts. It is marked by neurodegeneration and an accumulation of lipofuscin in neuronal tissues. Most cases of AD-ANCL do not present with visual abnormalities and their onset typically falls around the third to fourth decade of life. Furthermore, AD-ANCL is characterized by epileptic seizure disorders, motor dysfunction, and dementia (Bennett et al. 2013; Burgoyne and Morgan 2015).

In order to uncover more about the disease, it is imperative that one understands the role of cysteine-string protein alpha, as ANCL is caused by mutations in DNAJC5, which encodes this protein. CSP $\alpha$  is a synaptic co-chaperone found both in the cytosol and in lysosomal membranes, shown to exhibit anti-neurodegenerative qualities. Its role in membrane trafficking (both exocytosis and endocytosis) and protein folding appear to be critical for synaptic maintenance. Recall that the SNARE-complex is essential in the fusion of synaptic vesicles during exocytosis. Without the formation of the SNARE-complex, vesicles would not fuse with the presynaptic membrane, neurotransmitters would not be released into the synaptic cleft, and neuronal signaling would cease. Furthermore, the presynapse is able to endocytose extracellular content, allowing it to recycle certain proteins involved in neurotransmitter exocytosis and synaptic transmission. Improper functioning of these pathways is likely to result in neurodegeneration, as a lack of synaptic transmission could lead to a multitude of issues.

## **The Cysteine String Protein**

### *Discovery, Conservation, and Expression*

Cysteine-string protein (CSP) is an evolutionarily conserved protein discovered in a study of the *Drosophila* synapse (Zinsmaier et al. 1990). Following its discovery in flies, gene homologs to CSP were discovered in mammalian neuronal tissues, implying its conservation in all animals (Gunderson et al. 1994; Mastrogiacomo et al. 1995; Coppola and Gundersen 1996). CSP is found in both mammals and *Drosophila*, with minor but notable variations between the two. Three different genes encode mammalian CSP, producing the  $\alpha$ ,  $\beta$ , and  $\gamma$  isoforms, while *Drosophila* CSP is encoded by one gene

and has four different protein isoforms (Zinsmaier et al. 1994; Eberle et al. 1998). Nonetheless, the CSP $\alpha$  found in mammals is very similar to the *Drosophila* form, sharing an extensive portion of homology in the cysteine-string domain. CSP is composed of three conserved domains: the N-terminal J-domain joined to the cysteine-string domain, which are joined together by a third “linker” domain. Following the cysteine-string motif is a C-terminal region, which shows negligible homology to other isoforms of CSP (Zinsmaier et al. 1990). The N-terminus was given its name because of its homology to the DnaJ/Heat Shock Protein of the HSP40 gene family. This gene family interacts with Heat Shock Proteins of 70kD (HSP70), a family known for its ATPase function, which displays expression in the presence of stress factors such as heat (Craig et al. 1985; Lindquist 1988; Kiang 1998). The N-terminus also contains a conserved site of phosphorylation, which seems to be particularly important during synapse formation (Evans et al. 2003).

Although CSP $\alpha$  was discovered in the neuronal synapse, further investigation revealed its presence in both neuronal and non-neuronal cells. CSP $\alpha$  is abundant in neuropil regions of the brain, in which about 1% of the total synaptic vesicle protein is accounted for by CSP $\alpha$  (Zinsmaier 1990; Mastrogiacomo et al. 1994; Kohan et al. 1995). Neuropils are areas of the brain in which nervous tissue is very dense, consisting of many synapses. White matter tracts, on the other hand, show very little expression of CSP $\alpha$  (Kohan et al. 1995). CSP $\alpha$  is not only found in the synapse between neuronal cells, it is also found at the neuromuscular junction. In addition, CSP $\alpha$  can be found in secretory vesicles of the exocrine, endocrine, and neuroendocrine cells as well as tissues of the adrenal gland, kidney, liver, pancreas, spleen, and lung. The cells of the retina,

hippocampus, cerebellum, and many other areas also contain CSP $\alpha$ , showing its truly widespread nature (Kohan et al. 1995; Braun and Scheller 1995; Coppola and Gundersen 1996; Chamberlain and Burgoyne 1996; Zhao et al. 1997; Brown et al. 1998; Zhang et al. 1998; Eberle et al. 1998). Furthermore, as with most synaptic vesicle proteins, the amount of CSP $\alpha$  found at the synapse increases 10-20 fold throughout development into adulthood. However, studies have shown that the amount of phosphorylated CSP $\alpha$  only increases 4-5 fold, suggesting an important role of CSP $\alpha$  in synapse formation (Evans and Morgan 2005).

#### *Early Findings Regarding CSP's Role in Neuroprotection*

In order to understand the role of CSP $\alpha$ , genetic studies have been performed using flies, worms, and mice as animal models. Throughout the years, many relevant discoveries regarding CSP's neuroprotective role in synaptic maintenance have been made with the help of these animal models (Umbach et al. 1994; Zinsmaier et al. 1994; Heckmann et al. 1997; Umbach and Gundersen 1997; Eberle et al. 1998; Ranjan et al. 1998; Umbach et al. 1998; Dawson-Scully et al. 2000; Bronk et al. 2001; Fernandez-Chacon et al. 2004; Bronk et al. 2005; Chandra et al. 2005; Dawson-Scully et al. 2007).

*Drosophila* can be used as one of the more efficient models for studying CSP as their genome is nearly synonymous to the human genome and their lifecycle of about one month provides a convenient means of studying neuronal development throughout the lifecycle. To understand the role of CSP, *Drosophila* studies have both knocked out the CSP gene completely (Zinsmaier et al. 1994; Eberle et al. 1998; Fernandez-Chacon et al.

2004; Chandra et al. 2005) and overexpressed the gene (Nie et al. 1999) with surprisingly similar temperature-sensitive paralysis and lethality.

*Drosophila* lifespan and development is significantly affected by temperature. Many experiments involving *Drosophila* take place at room temperature. Increasing the temperature in which the fly vials are stored (i.e. 30°C) will speed up development, while decreasing the temperature (i.e. 11°C) will slow *Drosophila* activity and development (Roote and Prokop 2013). In experiments studying deletion of the CSP gene from the fly genome with development at room temperature, only 4% of the expected flies survived to adulthood. Of those who did survive, all exhibited progressive neurodegeneration and temperature-sensitive lethality in the form of “sluggishness, spasmic jumping, shaking, and uncoordinated locomotion ending in paralysis and premature death” (Umbach 1994; Zinsmaier et al. 1994; Eberle et al. 1998; Umbach et al. 1998).

When dCSP was overexpressed, *Drosophila* again displayed temperature-sensitive lethality. The few flies to survive to adulthood were observed only below 25°C, indicating even greater lethality with overexpression of CSP (Nie et al. 1999). Further studies hypothesized that CSP plays an important role in neurotransmitter release, such as a possible temperature-sensitive inhibition of Ca<sup>2+</sup> to enter the cell in *Drosophila* CSP mutants, thus preventing neuromuscular transmission (Heckmann et al. 1997; Umbach and Gundersen 1997; Ranjan et al. 1998; Umbach et al. 1998). However, later studies found that CSP mutants displayed a reduction in neurotransmitter release, even with an increase in Ca<sup>2+</sup> signaling in presynaptic terminals. This finding suggested CSP's role in downstream neurotransmitter release, possibly by increasing calcium ion sensitivity rather than regulating Ca<sup>2+</sup> entry (Dawson-Scully et al. 2000).

Studying CSP expression in mice has its own benefits. Mice have a more prolonged lifespan and as such, their development is more comparable to human development. When observing CSP $\alpha$  KO mice, no major phenotype of neurodegeneration was noted until about 2-4 weeks of life. Developmental defects and sensorimotor impairments were the first symptoms to appear around the second month of life (Fernandez-Chacon et al. 2004). This progressive neurodegeneration eventually resulted in blindness and paralysis and ended in premature death (Chandra et al. 2005; Schmitz et al. 2006). No inhibition of Ca<sup>2+</sup> influx or neurotransmitter release was noted in these studies. However, sensorimotor defects with marked neurodegeneration at the NMJ suggested CSP's role as a presynaptic chaperone with a neuroprotective role (Fernandez-Chacon 2004). Electromyographic studies of the neuromuscular junction in CSP $\alpha$  KO mice, showed an age-dependent degeneration at this synapse. In the first two weeks of life, NMJ function was normal with subsequent deterioration progressing through the later weeks of life (Fernandez-Chacon et al. 2004). Furthermore, observation of the NMJ using light and electron microscopy revealed underdeveloped postsynaptic areas, postsynaptic regions unoccupied by presynaptic neurons, and the presence of vacuoles and/or multilamellar bodies in the terminals, characteristic of degeneration and faulty neuronal trafficking (Fernandez-Chacon et al. 2004; Rozas et al. 2012). The analogous nature of disease phenotype and chronology observed in models of CSP $\alpha$  KO flies and mice is indicative of CSP's evolutionary conservation from *Drosophila* to *Mus Musculus* (mice).

Subsequent studies of CSP's function confirmed that CSP $\alpha$  KO *Drosophila* displayed no impairment in Ca<sup>2+</sup> influx, however CSP was shown to play a role in

regulating  $\text{Ca}^{2+}$  clearance as well as evoked release (and its thermal regulation/protection) and synaptic growth (Dawson-Scully et al. 2000; Bronk et al. 2005). Studies of the *Drosophila* NMJ in CSP $\alpha$  mutants found a decrease in neurotransmitter release in addition to a reduced number of boutons of specific motor neurons. However, there was no reduction in the number of synapses per bouton. The decrease in NT release was not correlated to a decrease in synapses, suggesting CSP's roles in several pathways independent of one another (Dawson-Scully et al. 2006)

### *Palmitoylation of CSP*

Palmitoylation is the process of adding the lipid palmitate to a cysteine residue and can be referred to as S-palmitoylation or N-palmitoylation depending on the method by which this 16-carbon saturated fatty acid is attached. If palmitate is added through a reversible thioester linkage the process is termed S-palmitoylation, while N-palmitoylation involves attachment at the N-terminus forming a stable amide linkage. Although the role of palmitoylation in ANCL is not yet completely understood, studies have associated this process with protein localization to specific membrane compartments and trafficking of proteins between organelles (Linder and Deschenes 2007). Within the neuron, palmitoylation seems to play a special role in protein sorting by working with other signals involved in membrane targeting to localize neuronal proteins to certain vesicles and the synaptic membrane (Huang et al. 2005). Furthermore, while palmitoylation of SNAP-25 is not required for membrane targeting, it is required for SNARE-complex dissociation and vesicle exocytosis regulation (Washbourne et al. 2005). Thus, palmitoylation is extremely important for neurotransmission among other

cellular processes. Unlike most post-translational lipid modifications, palmitoylation is reversible. As such, cyclical rounds of lipidation and de-lipidation are common of many palmitoylated proteins, including SNAP-25 and the oncogenic Ras small GTPases (Linder and Deschenes 2007; Fukata and Fukata 2010; Greaves and Chamberlain 2011).

Cysteine-string protein alpha's domain of 14 cysteine residues (the cysteine-string motif, after which the protein was named) is a highly lipidated region (Buchner and Gundersen 1997). By studying the *Torpedo* homolog of *Drosophila* CSPs, it was concluded that post-translational S-palmitoylation occurs on the vast majority of these cysteine residues (Gundersen et al. 1994). In the absence of palmitoylation, many studies found that CSP was not properly targeted to vesicles and was instead targeted to the endoplasmic reticulum membrane. These findings suggest the importance of the cysteine-string domain and its palmitoylation in secretion of CSP from the ER and in vesicular membrane targeting (Chamberlain and Burgoyne 1998; Arnold et al. 2004; Greaves and Chamberlain 2006).

There are 20 enzymes of the DHHC family located in intracellular membranes throughout the cell, which function as palmitoyl transferases (PAT) in mammalian intracellular membranes (Linder and Deschenes 2007). DHHC3, DHHC7, DHHC15, and DHHC17 are all located on the membrane of the Golgi and are the only mammalian PATs known to palmitoylate CSP (Greaves et al. 2008). One proposed hypothesis regarding the mechanism of this palmitoylation is that the cysteine-string motif of CSP is able to transiently associate with membranes due to its slight membrane affinity. Eventually CSP will encounter DHHC3, DHHC7, DHHC15, or DHHC17 on the Golgi membrane, at which point it will be palmitoylated and strongly associated with the

membrane. From there it will be trafficked through the Golgi until it is embedded in a vesicular membrane (Greaves et al. 2008).

DHHC17's *Drosophila* homolog Huntingtin-interacting protein 14 (Hip14, associated with Huntington's disease in its mutated form) has been the focus of several CSP palmitoylation studies, as it palmitoylates CSP in *Drosophila* neuronal tissues, suggesting a resemblance between CLN4 and Huntington's disease (Ohyama et al. 2007; Stowers and Isacoff 2007; Henderson 2016). These studies involving Hip14 mutants showed analogous results to CSP mutant studies in which neurotransmitter release showed progressive deficit at higher temperatures until the complete loss of evoked release was reached (Ohyama et al. 2007). This evidence is implicit of the necessity for CSP palmitoylation, as it permits synaptic vesicle association and functional synaptic transmission.

Notably, there is very little evidence supporting the lipidation and de-lipidation of CSP, characteristic of most palmitoylated proteins. Some studies have suggested that CSP does undergo this process in order to associate into the membrane via "acyl-flip" or "bilayer collapse" models but with very little supporting evidence (Gundersen et al. 1995; Zinsmaier and Imad 2011). However, recent evidence suggests that CSP $\alpha$  may be targeted by PPT1 (protein palmitoyl thioesterase), a depalmitoylating enzyme implicated in the disease progression caused by CLN1 mutations, which have both infantile and adult-onset forms (Carcel-Trullols et al. 2015; Henderson et al. 2016).

### *DnaJ, CSP $\alpha$ , and Hsc70*

DNAJC5, of the evolutionarily conserved DnaJ gene family, encodes cysteine string proteins and as such, all CSP's have a J domain (Chamberlain and Burgoyne 1997a). DnaJ commonly interacts with the chaperone protein DnaK in bacterial populations. Similarly, CSP's J-domain is responsible for its interaction with a 70-kDa heat-shock protein (Hsp70), the homologue of DnaK in eukaryotes (Caplan 1993; Silver 1993; Chamberlain and Burgoyne 1997). Hsp70 is a family of stress-induced chaperone proteins with an ATPase activity permitting its transiency in protein interactions (Chamberlain and Burgoyne 1997a; Bukau 1998; Tobaben 2003).

One member of the Hsp70 protein family particularly important to this discussion is Hsc70, a 70-kDa heat-shock cognate protein. Auxilin is a protein cofactor of Hsc70, which binds to clathrin-coated vesicles and recruits Hsc70 (Chamberlain and Burgoyne 1997a). Clathrin is a protein coating endocytosed vesicles and vesicles moving through the Golgi in order to assist these vesicles in budding from their respective donor membranes (Royle 2006). Once the vesicle buds off the membrane, it no longer needs its clathrin coating. When Hsc70 is recruited to clathrin-coated vesicles, it un-coats these vesicles in an ATP-dependent process so they may become early endosomes (Chamberlain and Burgoyne 1997a). Auxilin and Hsc70 seem to function together in other important processes as well, such as preventing clathrin aggregation in the cell after un-coating and aiding clathrin in its reattachment to the plasma membrane so it may be used again in the formation of new clathrin-coated pits (Eisenberg and Greene 2007).

DnaJ interacts with Hsp70 to play several central roles in the cell including un-coating clathrin-coated vesicles, refolding misfolded proteins, and degrading damaged or

excess proteins through ubiquitination. The mechanism by which these processes typically take place begins with DnaJ binding to its substrate (typically a misfolded or aggregated protein), which then binds to Hsp70-ATP. Hsp70 then hydrolyzes ATP to ADP, thus creating a stronger affinity and allowing binding between DnaJ, substrate, and Hsp70. From there, the substrate is either ubiquitinated (marked for degradation) or refolded via a nucleotide-exchange process and released back into the cell (Kampinga and Craig 2010).

In vitro analysis combining bovine Hsc70 with CSP shows the activation of Hsc70's ATPase activity and complex-formation between the two proteins (Braun et al. 1996; Chamberlain and Burgoyne 1997). The association between CSP and Hsc70 is implicit of Hsc70's critical role in exocytosis, supported by the significant decrease in neurotransmitter release seen in *Drosophila* Hsc70 mutants (Bronk et al. 2001). Many possible substrates of CSP have been suggested thus far, yet the significance of these interactions utilizing Hsc70-ATP is largely unknown. Studies have shown that the cooperative interaction between CSP and Hsc70 works to prevent denatured protein aggregation in the cell. Thus, it is possible that CSP-Hsc70 prevents the aggregation of these proposed substrates such as synaptobrevin, syntaxin, and the Ca<sup>2+</sup> sensor, synaptotagmin (Leveque et al. 1998; Wu et al. 1999; Nie et al. 1999; Evans and Morgan 2002). Many of the potential clients of CSP are proteins of the SNARE-complex involved in exocytosis, such as syntaxin, synaptobrevin, and SNAP-25, suggesting CSP's critical role in neurotransmission.

### *CSP $\alpha$ and the SNARE-Complex*

Studies of the cysteine-string protein and its involvement in AD-ANCL have provided evidence that CSP $\alpha$  may prevent neurodegeneration by maintaining the conformation of SNAP-25. Accordingly, wild-type CSP $\alpha$  would seem to play an indirect role in the facilitation of the correct SNARE-complex formation. Experiments involving CSP $\alpha$  KO mice revealed a significant reduction in synaptic SNAP-25 levels and thus a reduction in SNARE-complexes (Chandra et al. 2005). When CSP $\alpha$  levels are decreased due to mutations in DNAJC5, SNAP-25 is improperly folded and consequently ubiquitinated leading to its proteolysis in the lysosome (Chandra et al. 2005; Sharma et al. 2011). Furthermore, studies have shown that overexpression of CSP $\alpha$  yields a correlated increase in SNAP-25 levels at the synapse (Zhang et al. 2012). In studies of CSP $\alpha$  KO mice aimed at expanding our understanding of this correlation, it was elucidated that overexpression of SNAP-25 greatly reduced neurodegenerative findings, while partial deletion of SNAP-25 from the genome (heterozygous KO) intensified neurodegeneration in addition to decreasing SNARE- complex formation and further reducing lifespan (Sharma et al. 2012). These findings provide further evidence of the correlation between CSP $\alpha$  and SNAP-25 and their combined involvement in neurodegeneration. One experiment challenging this theory of ANCL pathogenesis due to a reduction in SNAP-25 reported that a knockdown (KD) of SNAP-25 in wild-type neurons did not show a large decrease in synaptic transmission (Sharma et al. 2012). These results indicate that the decreased level of functional SNAP-25 plays a large role in ANCL neurodegeneration. However, this is only one of several contributing factors to a very complicated disease. The loss of functional CSP $\alpha$  has many negative impacts on the

synapse that, in conjunction with one another, lead to this aggressive and complex disorder.

Studies suggest that CSP $\alpha$  interacts with its clients such as SNAP-25 through a trimeric chaperone complex with Hsc70 and SGT (small glutamine-rich tetratricopeptide repeat protein) in the formation of the SNARE-complex (Tobaben et al. 2001; Tobaben et al. 2003; Sharma et al. 2011; Cadieux-Dion 2013). Through biochemical analysis, CSP $\alpha$  was demonstrated to recruit, bind to, and activate Hsc70's ATPase function, leading to the activation of its clients. However, this same study highlighted the discrepancy in the hypothesis regarding the CSP $\alpha$ -Hsc70-SGT complex, as all of this data has come from in vitro studies rather than in vivo studies with palmitoylated CSP $\alpha$  (Burgoyne et al. 2015).

In mutant and knockout CSP $\alpha$  animal models, neurodegeneration seems to be caused by defective SNAP-25 and other SNARE binding proteins. In studies of deceased Alzheimer's patients, post-mortem brain tissue samples have shown a marked decrease in CSP $\alpha$  in degenerative neuronal tissues, while CSP $\alpha$  is surprisingly increased in non-degenerative areas compared to healthy subjects (Tiwari et al. 2015). Therefore, it would seem that the same distribution of CSP $\alpha$  mutants might be seen in ANCL, providing a fair representation of ANCL disease phenotype. One study found that when CSP $\alpha$  was eliminated from neuronal tissues in mice models, there was a 59% reduction in SNAP-25, 41% reduction in synaptobrevin levels, 43% reduction in syntaxin levels, and no changes in CSP's partner Hsc70 (Benitez, 2015). These were only some of the proteins demonstrating altered expression patterns as a result of abnormal CSP $\alpha$ . This implies that in the absence of CSP $\alpha$  many SNARE proteins do not form properly. Thus, CSP $\alpha$  dysfunction seems to inhibit lysosomal fusion events (i.e. the SNARE-complex), leading

to the formation of ceroid aggregates and the subsequent swelling of the cellular soma, characteristic of NCL.

The correlation between SNAP-25 and CSP $\alpha$  is a relevant area of confusion due to their relative locations and abundance. SNAP-25 is largely localized in areas other than the active zone of CSP $\alpha$  binding and SNAP-25 is in much higher abundance (more than 25-fold in excess) than CSP $\alpha$  (Takamori et al. 2006; Willhelm et al. 2014; Burgoyne and Morgan 2015). This discrepancy could be understood as an evolutionary mechanism to ensure energy conservation. If the energy associated with the hydrolysis of ATP by Hsc70 is only expended on SNAP-25 molecules capable of forming a local SNARE-complex, this would provide an energetically favorable mechanism for membrane fusion in that area. If this hypothesis were true, it would prevent Hsc70 (in cooperation with CSP $\alpha$ ) from expending energy on SNAP-25 molecules that will not be involved in a SNARE-complex. Whether this hypothesis is true still remains to be elucidated leaving a significant gap in the to-date knowledge of ANCL disease pathology.

Certain studies have attempted to elucidate a connection between the synuclein gene family, involved in Parkinson's, and CSP $\alpha$ . The synucleins are comprised of three genes ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -synuclein) and their functions remain uncertain, although evidence suggests they play a role in presynaptic membrane-associated processes (George 2002). In a study of CSP $\alpha$  KO mice, overexpression of  $\alpha$ -synuclein drastically reversed ANCL neurodegeneration and lethality (Sharma et al. 2011; Ruiz et al. 2014). Furthermore, removal of  $\alpha$ -synuclein in CSP $\alpha$  KO mice worsened neurodegeneration and reduced SNARE-complex formation (Chandra et al. 2005). In  $\alpha\beta\gamma$ -synuclein KO mice with wild-type CSP $\alpha$ , results showed an up-regulation in CSP $\alpha$  as a compensatory mechanism for

the reduction in SNARE-complex formation (Burre et al. 2010). Even mutated forms of  $\alpha$ -synuclein (A30P mutants) known to cause pathogenesis revealed a neuroprotective function at the synapse (Ruiz et al. 2014). Prior to this finding, the synaptic vesicle proteins CSP $\alpha$  and  $\alpha$ -synuclein were both known to be involved in neurodegeneration (one in ANCL and the other in Parkinson's, respectively), however the functions of these proteins in the cell were assumed to be completely independent of one another. Although the overexpression of  $\alpha$ -synuclein reversed SNARE-complex reduction and neurodegeneration, it is important to note that it did not reverse the depletion of synaptic SNAP-25, characteristic of CSP $\alpha$  KO mice. This finding may explain the previously explained absence of neurodegeneration comparable to ANCL in wild-type CSP $\alpha$  SNAP-25 knockdown neurons (Sharma et al. 2012). Also of relevance is the finding that  $\alpha$ -synuclein does not display any interaction with CSP $\alpha$ 's co-chaperones SGT and Hsc70. Perhaps other proteins associated with neurotransmitter release, such as  $\alpha$ -synuclein, rescue a lack of SNAP-25 in otherwise healthy neurons. This would imply that the two proteins have similar roles in synaptic transmission and neuroprotection.

### *CSP $\alpha$ and Dynamin*

In contrast to SNARE proteins, which are vital for the fusion of a vesicle with the cell membrane in exocytosis, the dynamin family consists of proteins involved in just the opposite: budding and scission of an incoming vesicle for endocytosis. Although CSP $\alpha$ 's role in exocytosis has been extensively studied, its function in regulating endocytosis was discovered relatively recently. It seems that, just as CSP $\alpha$  is vital for normal, functional levels of several SNARE proteins, CSP $\alpha$  also regulates dynamin-1 levels in the cell and

thus has a critical function in endocytosis. Dynamin-1 is a GTPase mainly localized in the presynapse of brain neurons. This protein mediates endocytosis by forming oligomers on either side of the vesicular neck and then uses its GTPase function to hydrolyze and separate the budding vesicle from the cell membrane (Sweitzer and Hindshaw 1998). Quantifications of synaptic proteins in CSP $\alpha$  KO mice revealed a decrease in dynamin-1 (among other proteins, such as SNAP-25) prior to ANCL phenotype emergence, suggesting a causal relationship between CSP $\alpha$ -dependent protein levels and impending neurodegeneration. In the same study, pull-down assays of wild-type mice synaptic proteins with CSP $\alpha$  and Hsc70 were performed, in which nucleotide-dependent binding between CSP $\alpha$  and dynamin-1 was discovered, similar to the ADP-dependent binding between Hsc70 and CSP $\alpha$ . In these pull-down assays, SNAP-25 bound to CSP $\alpha$  and Hsc70 as expected, but dynamin-1 did not bind Hsc70 (Zhang et al. 2012). Further investigations revealed that wild type and mutant CSP $\alpha$  mice brain homogenates bind SNAP-25 and dynamin-1 with equal affinity when CSP $\alpha$  is not oligomerized. This finding is consistent with CSP $\alpha$ 's role as a co-chaperone for SNAP-25 and dynamin (Zhang and Chandra 2014).

## **Adult-Onset Neuronal Ceroid Lipofuscinosis**

### *A Brief History of ANCL*

The first known case of ANCL was described in 1971 in several members of the Parry family. Therefore Kufs Disease (AD-ANCL) is often referred to as Parry Disease, since this was its original identification (Boehme et al. 1971; Velinov 2012). All members of the Parry family affected with the disease presented with similar clinical

findings and chronology including onset in the late 20's or 30's, epilepsy, visual disturbances, short-term memory decline, sensorimotor impairments, premature death, and the histological finding of autofluorescent pigment in neuronal tissues (Boehme et al. 1971).

Following the initial identification of Kufs disease, other cases of ANCL arose with autosomal-dominant inheritance and very similar clinical and histological presentation, although most of these subjects did not suffer from visual impairment (Noskova et al. 2011). Electron microscopy studies revealed lysosomal inclusions specific to NCL, such as granular osmophilic deposits (GROD) and the accumulation of saposin D, involved in the activation of lysosomal hydrolytic enzymes. These findings, in conjunction with normal PPT1 levels, suggested that Kufs Disease was linked to a gene unassociated with NCL at that time (Nijssen et al. 2003).

Similar aggregates and accumulations of GRODs and other autofluorescent lipofuscin pigments are noted in all known forms of the disease. Interestingly, many studies have automatically classified these aggregates as pathological. However, certain cases have shown that one may have the disease with accumulations of lipofuscin similar to that of end-stage patients, with no symptom-onset (Benitez et al. 2015). Therefore it is impossible to say whether these aggregates actually cause toxicity or are simply a corollary clinical finding of the disease with completely arbitrary effects.

### *Mutations Linked to AD-ANCL*

Two mutations linked to AD-ANCL have been identified in DNAJC5 causing pathological alterations in the cysteine-string motif of CSP $\alpha$ . The cysteine-string motif is a critical region enabling CSP $\alpha$  trafficking to vesicular membranes in its palmitoylated form, making it no surprise that mutation in this region could have pathological results. L116 $\Delta$  is one of these mutations, which denotes the in-frame deletion of an amino acid within the motif. Genotyping studies of two different families with ANCL suggested that this mutation is recurrent, rather than a founder mutation, due to its presence on two distinct haplotypes (Cadieux-Dion et al. 2013). Since one mutation led to the same disease in two unrelated families, the study concluded that ANCL mutations are inherited and do not spontaneously arise. The other mutation causative of AD-ANCL is L115R, a heterozygous point mutation in which a leucine amino acid is replaced with arginine (Benitez et al. 2011; Greaves et al. 2012; Velinov et al. 2012; Cadieux-Dion et al. 2013).

Both mutations were hypothesized to inhibit palmitoylation of the cysteine-string motif, thereby impairing proper CSP $\alpha$  trafficking, possibly due to the loss of a dileucine motif or a decrease in overall hydrophobicity. The L115R mutation swaps an amino acid for another with very different chemical properties, as leucine is nonpolar and arginine is polar and positively charged. Thus, it came as no surprise that the L115R mutated CSP $\alpha$  exhibited a decrease in overall hydrophobicity and impaired the palmitoylation of cysteine residues in several *in silico* analyses (Noskova et al. 2011; Benitez et al. 2011).

Migration profiles on SDS gels of the CSP $\alpha$  mutation L115A, in which leucine is swapped for the hydrophobic amino acid, alanine, revealed similar migration to that of wild type CSP $\alpha$ . This provides further evidence that the neurodegeneration caused by

L115R and L116 $\Delta$  mutants is due to a loss of hydrophobicity rather than being caused by the loss of a dileucine motif (Greaves et al 2012). It is important to note that while some studies did observe a decrease in palmitoylation of cysteine residues adjacent to the L116 $\Delta$  region (Noskova et al. 2011), others did not (Benitez et al. 2011). However it is possible that these studies may not have used the most reliable methods.

In comparisons between wild type and mutant CSP $\alpha$  biochemical properties, many interesting observations have been made. Studies have revealed a significant reduction of CSP $\alpha$  in both L116 $\Delta$  and L115R mutant post-mortem human neuronal tissue (Noskova et al. 2011). Furthermore, while wild type CSP $\alpha$  is localized to the synaptic vesicle membrane, both L115R and L116 $\Delta$  mutants appear to be largely mistargeted upon confocal microscopy of EGFP-tagged neuroendocrine PC12 cells. This same study also concluded that the mutant aggregates of CSP $\alpha$  were SDS-resistant, characteristic of other proteins involved in neurodegeneration. Aggregation was induced when CSP $\alpha$  was co-expressed with palmitoyltransferase enzymes, while depalmitoylation enzyme co-expression caused the aggregates to break up. This finding suggests that the SDS-resistant aggregates are palmitoylation-dependent and that aggregation is reversible (Greaves et al. 2012). However, the palmitoylation-dependence of these aggregates may be subject to questioning, the results could have been induced by excessive overexpression of palmitoyltransferases creating an unrealistic model of in vivo disease (Zhang and Chandra 2014).

A later study by Zhang and Chandra (2014) yielded results contrary to Greaves et al. (2012) in an investigation of *E. Coli* CSP $\alpha$ , a species that does not possess palmitoyltransferases similar to the human DHHC enzymes. These CSP $\alpha$  mutants had a

much higher tendency to form aggregates than wild type CSP $\alpha$ , indicating palmitoylation is unnecessary for oligomerization to occur. These aggregates were formed in a time, concentration, and temperature-dependent manner. The L115R mutants displayed a lower overall concentration and a higher tendency to oligomerize than L116 $\Delta$  in patient brains. These distinctions, in addition to L116 $\Delta$ 's higher affinity to co-oligomerize with wild type CSP $\alpha$ , indicate the potential for subtle differences in disease pathophysiology and chronology between L115R and L116 $\Delta$ . SGT did not seem to play a role in CSP $\alpha$  oligomerization of ANCL mutants either, suggesting that mutant CSP $\alpha$  has an intrinsic ability to form aggregates (Zhang and Chandra 2014). Both mutations form a 250-kDa oligomeric species, which may be of relevance to disease pathology. The study revealed the selective ubiquitination of 250-kDa oligomers of CSP $\alpha$ , leading to their hypothesis that a high level of ubiquitinated mutant protein oligomers leads to lysosomal dysfunction as the lysosomes constantly work to break down these oligomers over time (Zhang and Chandra 2014).

Moreover, CSP $\alpha$  mutants were able to activate Hsc70's ATPase activity to the same degree as wild type CSP $\alpha$  indicating a functional J-domain in mutant proteins. Affinity chromatography was then used to study the ability of mutant CSP $\alpha$  to bind clients such as dynamin and SNAP-25. They found that mutant CSP $\alpha$  binds its clients as effectively as its wild type counterpart, indicating a functional C-terminal domain (Zhang and Chandra 2014). Thus, the loss of mutant CSP $\alpha$ 's co-chaperone function is likely due to oligomerization leading to a loss of CSP $\alpha$  monomers available to chaperone in the cell. The same study observed the loss of palmitoylated CSP $\alpha$  monomers in both mutant forms, likely due to mistargeting of CSP $\alpha$  (Greaves et al. 2006; Zhang and Chandra

2014). This study was an incredible breakthrough in the current understanding of CSP $\alpha$  mutants, as the findings implied that ANCL is caused by gain-of-function mutations leading to a loss-of-function. The gain of function described here is oligomerization, while the loss of CSP $\alpha$  chaperone activity is dependent on oligomerization. Similar to other autosomal dominant neurodegenerative diseases, these findings highlight the complicated nature of ANCL, its pathophysiology caused by the summation of several different mechanisms (Zhang and Chandra 2014).

In a study of post-mortem ANCL patient brains, several important observations were made. The first was that although CSP $\alpha$  mRNA levels were normal, CSP $\alpha$  protein levels were greatly reduced, especially in L115R mutants (Henderson et al. 2016). Second, this study revealed a 90-fold increase and mis-localization of PPT1 (the depalmitoylating enzyme mutated in CLN1) in DNAJC5/CLN4 patient brains. The PPT1 in these neuronal tissues, despite the observed quantitative increase, displayed a surprisingly high reduction in enzymatic activity. Nonetheless, using immunoprecipitated CSP $\alpha$  and human PPT1 for in vitro depalmitoylation assays yielded depalmitoylated CSP $\alpha$ , implicit of a substrate-enzyme relationship between CSP $\alpha$  and PPT1 (Henderson et al. 2016). Furthermore, a lysosomal enzyme known to cause CLN10 in its mutated form, Cathepsin D, was increased 10-fold. This, along with an accumulation of Saposin A and D observed in all three subtypes (CLN1, CLN4, and CLN10), suggests a possible interaction between NCL genes or, perhaps, that various forms of NCL share some pathological mechanisms in common.

Due to the oligomerization of CSP $\alpha$  seen in CLN4 patient brains, it is possible that the striking increase in PPT1 could be a cellular defense in its attempt to degrade

CSP $\alpha$  mutants. Over time, PPT1 would accumulate in the cell and eventually exhibit altered functioning dependent on location (Henderson et al. 2016). The accumulation of these irregularities in the cell causes abnormal palmitoylation of mutant CSP $\alpha$ - a possible causative factor of CLN4 neurodegeneration.

Many of the studies investigating ultrastructural and clinical features of ANCL have used post-mortem ANCL patient brains, which were in the terminal stages of the disease with tremendous neurodegeneration and significantly reduced levels of CSP $\alpha$ . However, a recent study was able to investigate the neuronal cells of an early-stage patient whose premature death at age 37 was caused by a motor vehicle accident (Benitez et al. 2015). This patient had the L115R mutation of DNAJC5/CSP $\alpha$  and had a family history of ANCL. The study revealed the presence of AFSM in neuronal cell bodies, most likely related to the lysosome (Benitez et al. 2015). Despite the fact that this patient had not yet experienced any major symptoms of ANCL and exhibited no brain atrophy or decreased levels of CSP $\alpha$ , these histopathological findings were essentially indistinguishable from terminal patients (Benitez et al. 2015). This discovery suggests that lysosomal dysfunction precedes neurodegeneration caused by toxic aggregates of CSP $\alpha$ .

### III. Concluding Remarks

The papers reviewed in this thesis bring together the current state of knowledge surrounding ANCL and CSP $\alpha$ . Research on this topic will likely be continued for some time to come, as it is still unclear exactly what pathological mechanisms underlie ANCL. However, from the discovery of CSP $\alpha$  to now, many important insights have given us a more detailed picture of ANCL.

Recent findings challenge the prior assumption that ANCL is caused by loss-of-function mutations, as studies now show that CSP $\alpha$  gains the function of oligomerization. Still, oligomerization ultimately leads to impaired synaptic transmission and thus ANCL is caused by gain-of-function mutations instigating a loss-of-function at the synapse (Zhang and Chandra 2014; Henderson et al. 2016). Furthermore, the current literature suggests that L115R and L116 $\Delta$  mutations are inherited in a dominant-negative fashion, inhibiting the normal functions of CSP $\alpha$  and possibly PPT1 (Noskova et al. 2011; Greaves et al. 2012; Henderson et al. 2016). This seems to be the most commonly proposed hypothesis in the realm of ANCL research at the present time.

Previous studies have focused extensively on the presence of autofluorescent ceroid-lipofuscin aggregates in ANCL subjects, as it seemed this might explain disease pathology. However, studies have not yet found any effect from these aggregates on neurodegeneration, suggesting they are simply a correlated clinical finding. Supporting this hypothesis is the study of an early-stage ANCL subject involved in a motor vehicle accident, who displayed neuronal lipofuscin aggregates prior to symptom onset (Benitez et al. 2015). This is evidence of the propensity in scientific research to classify certain characteristics as pathological simply because they are not seen in normal, healthy cells.

However, whether these characteristics cause toxicity and therefore neurodegeneration remains to be proven.

Every new finding brings the challenging and enigmatic case of ANCL one step closer to being cracked. A combination of in vivo studies of animal models and studies of deceased ANCL patients has provided a great deal of knowledge to this topic and likely will continue to do so. Gaining a deeper understanding of ANCL pathology will likely generate an improved grasp on other neurodegenerative disorders as well as the potential ability to help those suffering from this disease.

## References

- Aby, E., Gumps, K., Roth, A., Sigmon, S., Jenkins, S.E., Kim, J.J., Kramer, N.J., Parfitt, K.D. and Korey, C.A. 2013. Mutations in palmitoyl-protein thioesterase 1 alter exocytosis and endocytosis at synapses in *Drosophila* larvae. *Fly* 7(4), pp. 267–279.
- Arnold, C., Reisch, N., Leibold, C., Becker, S., Prüfert, K., Sautter, K., Palm, D., Jatzke, S., Buchner, S. and Buchner, E. 2004. Structure-function analysis of the cysteine string protein in *Drosophila*: cysteine string, linker and C terminus. *The Journal of Experimental Biology* 207 (Pt 8), pp. 1323–1334.
- Arsov, T., Smith, K. R., Damiano, J., Franceschetti, S., Canafoglia, L., et al. (2011). Kufs disease, the major adult form of neuronal ceroid lipofuscinosis, caused by mutations in CLN6. *Am J Hum Genet* 88: 566–573.
- Benitez, B.A., Cairns, N.J., Schmidt, R.E., Morris, J.C., Norton, J.B., Cruchaga, C. and Sands, M.S. (2015). Clinically early-stage CSP $\alpha$  mutation carrier exhibits remarkable terminal stage neuronal pathology with minimal evidence of synaptic loss. *Acta neuropathologica communications* 3, p. 73.
- Bennett, M. J., & Rakheja, D. (2013). The neuronal ceroid-lipofuscinoses. *Developmental Disabilities Research Reviews*, 17(3), 254-259.
- Boehme, D. H., Cottrell, S. C., Leonberg, S. C., Zeman, W. (1971). A dominant form of Neuronal Ceroid Lipofuscinosis. *Brain* 94: 745–760.
- Braun, J.E. and Scheller, R.H. (1995). Cysteine string protein, a DnaJ family member, is present on diverse secretory vesicles. *Neuropharmacology* 34(11), pp. 1361-1369.
- Braun, J. E., Wilbanks, S. M., Scheller, R. H. (1996). The cysteine string secretory vesicle protein activates Hsc70 ATPase. *J. Biol. Chem.* 271: 25989-93.
- Bronk, P., Wenniger, J.J., Dawson-Scully, K., Guo, X., Hong, S., Atwood, H.L., Zinsmaier, K.E. (2001). *Drosophila* Hsc70–4 is critical for neurotransmitter exocytosis in vivo. *Neuron*. 30:475–488
- Bronk, P., Nie, Z., Klose, M.K., Dawson-Scully, K., Zhang, J., Robertson, R.M., Atwood, H.L. and Zinsmaier, K.E. (2005). The multiple functions of cysteine string protein analyzed at *Drosophila* nerve terminals. *The Journal of Neuroscience* 25(9), pp. 2204–2214.
- Brown, H., Larsson, O., Branstrom, R., Yang, S.N., Leibiger, B., Leibiger, I., Fried, G., Moede, T., Deeney, J.T., Brown, G.R., Jacobsson, G., Rhodes, C.J., Braun, J.E., Scheller, R.H., Corkey, B.E., Berggren, P.O., Meister, B. (1998). Cysteine string protein (CSP) is an insulin secretory granule-associated protein regulating beta-cell exocytosis. *EMBO J* 17:5048–5058.

- Buchner, E., Gundersen, C.B. (1997). The DnaJ-like cysteine string protein and exocytotic neurotransmitter release. *TINS* 20:223–227.
- Burgoyne, R.D. and Morgan, A. (2015). Cysteine string protein (CSP) and its role in preventing neurodegeneration. *Seminars in Cell & Developmental Biology* 40, pp. 153–159.
- Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R. and Südhof, T.C. (2010). Alpha synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science (New York)* 329(5999), pp. 1663–1667.
- Bukau, B., & Horwich, A. L. (1998). The Hsp70 and Hsp60 Chaperone Machines. *Cell*, 92(3), 351–366.
- Cadioux-Dion, M., Andermann, E., Lachance-Touchette, P., Ansorge, O., Meloche, C., Barnabé, A., Kuzniecky, R.I., Andermann, F., Faught, E., Leonberg, S., Damiano, J.A., Berkovic, S.F., Rouleau, G.A. and Cossette, P. (2013). Recurrent mutations in DNAJC5 cause autosomal dominant Kufs disease. *Clinical Genetics* 83(6), pp. 571–575.
- Caplan, A.J., Cyr, D.M., Douglas, M.G. (1993). Eukaryotic homologues of *Escherichia coli* dnaJ: a diverse protein family that functions with hsp70 stress proteins. *Mol Biol. Cell*, 4. 555–63.
- Cárcel-Trullols, J., Kovács, A.D. and Pearce, D.A. (2015). Cell biology of the NCL proteins: What they do and don't do. *Biochimica et Biophysica Acta* 1852(10 Pt B), pp. 2242–2255.
- Chamberlain, L.H., Burgoyne, R.D. (1996b). Identification of a novel cysteine string protein variant and expression of cysteine string proteins in non-neuronal cells. *J Biol Chem* 271:7320–7323.
- Chamberlain, L.H. and Burgoyne, R.D. (1997a). Activation of the ATPase activity of heat shock proteins Hsc70/Hsp70 by cysteine-string protein. *The Biochemical Journal* 322 ( Pt 3), pp. 853–858.
- Chamberlain, L.H., Burgoyne, R.D. (1998b). The cysteine-string domain of the secretory vesicle cysteine-string protein is required for membrane targeting. *Biochem J* 335:205–209.
- Chandra, S., Gallardo, G., Fernandez-Chacon, R., Schluter, O.M., Südhof, T.C. (2005). Alpha synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell* 123:383–396.
- Chau, V., Tobias, J., Bachmair, A., Marriott, D., Ecker, D., Gonda, D., & Varshavsky, A. (1989). A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science*, 243(4898), 1576–1583.
- Coppola, T., Gundersen, C. (1996). Widespread expression of human cysteine string proteins. *FEBS Lett* 391:269–272.

- Cotman, S. L., Karaa, A., Staropoli, J. F., & Sims, K. B. (2013). Neuronal Ceroid Lipofuscinosis: Impact of Recent Genetic Advances and Expansion of the Clinicopathologic Spectrum. *Current Neurology and Neuroscience Reports*, 13(8).
- Craig, E. A., & Jacobsen, K. (1985). Mutations in cognate genes of *Saccharomyces cerevisiae* hsp70 result in reduced growth rates at low temperatures. *Molecular and Cellular Biology*, 5(12), 3517-3524.
- Dawson-Scully, K., Bronk, P., Atwood, H.L., Zinsmaier, K.E. (2000). Cysteine-string protein increases the calcium sensitivity of neurotransmitter exocytosis in *Drosophila*. *J Neurosci* 20:6039-6047
- Dawson-Scully, K., Lin, Y., Imad, M., Zhang, J., Marin, L., Horne, J.A., Meinertzhagen, I.A., Karunanithi, S., Zinsmaier, K.E., Atwood, H.L. (2007). Morphological and functional effects of altered cysteine string protein at the *Drosophila* larval neuromuscular junction. *Synapse* 61:1-16.
- Eberle, K.K., Zinsmaier, K.E., Buchner, S.M.G., Jenni, M., Arnold, C., Leibold, C., Reisch, D., Walter, N., Hafen, E., Hofbauer, A., Pflugfelder, G.O., Buchner, E. (1998). Wide distribution of cysteine string protein in *Drosophila* tissues revealed by targeted mutagenesis. *Cell Tissue Res* 294:203-217.
- Eisenberg, E., Greene, L.E. (2007). Multiple roles of auxilin and hsc70 in clathrin-mediated endocytosis. *Traffic* 8:640-646.
- Evans, G.J., Morgan, A. (2002). Phosphorylation-dependent interaction of the synaptic vesicle proteins cysteine string protein and synaptotagmin I. *Biochem J* 364:343-347.
- Evans, G.J., Morgan, A. (2003). Regulation of the exocytotic machinery by cAMP-dependent protein kinase: implications for presynaptic plasticity. *Biochem Soc Trans* 31:824-827.
- Evans, G.J., Morgan, A. (2005). Phosphorylation of cysteine string protein in the brain: developmental, regional and synaptic specificity. *Eur J Neurosci* 21:2671-2680.
- Fernández-Chacón, R., Wölfel, M., Nishimune, H., Tabares, L., Schmitz, F., Castellano-Muñoz, M., Rosenmund, C., Montesinos, M.L., Sanes, J.R., Schneggenburger, R., Südhof, T. C. (2004). The Synaptic Vesicle Protein CSP $\alpha$  Prevents Presynaptic Degeneration. *Neuron*, 42(2), 237-251.
- Fontaine, S.N., Zheng, D., Sabbagh, J.J., Martin, M.D., Chaput, D., Darling, A., Trotter, J.H., Stothert, A.R., Nordhues, B.A., Lussier, A., Baker, J., Shelton, L., Kahn, M., Blair, L.J., Stevens, S.M. and Dickey, C.A. (2016). DnaJ/Hsc70 chaperone complexes control the extracellular release of neurodegenerative-associated proteins. *The EMBO Journal*.
- Franco, M., Seyfried, N. T., Brand, A. H., Peng, J., & Mayor, U. (2010). A Novel Strategy to Isolate Ubiquitin Conjugates Reveals Wide Role for Ubiquitination during Neural Development. *Molecular & Cellular Proteomics*, 10(5).
- Goldstein, L.S. (2003). Do disorders of movement cause movement disorders and dementia? *Neuron* 40:415-425.

- Greaves, J., Lemonidis, K., Gorleku, O.A., Cruchaga, C., Grefen, C. and Chamberlain, L.H. (2012). Palmitoylation-induced aggregation of cysteine-string protein mutants that cause neuronal ceroid lipofuscinosis. *The Journal of Biological Chemistry* 287(44), pp. 37330–37339.
- Greaves, J. and Chamberlain, L.H. (2006). Dual Role of the Cysteine-String Domain in Membrane Binding and Palmitoylation-dependent Sorting of the Molecular Chaperone Cysteine-String Protein. *Molecular biology of the cell* 17(11), pp. 4748–4759.
- Greaves, J. and Chamberlain, L.H. (2011). Differential palmitoylation regulates intracellular patterning of SNAP25. *Journal of Cell Science* 124(Pt 8), pp. 1351–1360.
- Gundersen, C.B., Mastrogiacomo, A., Faull, K. and Umbach, J.A. (1994). Extensive lipidation of a Torpedo cysteine string protein. *The Journal of Biological Chemistry* 269(30), pp. 19197–19199.
- Heckmann, M., Adelsberger, H., Dudel, J. (1997). Evoked transmitter release at neuromuscular junctions in wild type and cysteine string protein null mutant larvae of *Drosophila*. *Neurosci Lett* 228:167–170.
- Hellsten, E., Vesa, J., Olkkonen, V.M., Jalanko, A. and Peltonen, L. (1996). Human palmitoyl protein thioesterase: evidence for lysosomal targeting of the enzyme and disturbed cellular routing in infantile neuronal ceroid lipofuscinosis. *The EMBO Journal* 15(19), pp. 5240–5245.
- Hobert, J.A., Dawson, G. (2006). Neuronal ceroid lipofuscinoses therapeutic strategies: Past, present and future. *Biochem Biophys Acta* 1762: 945–953.
- Huang, K., & El-Husseini, A. (2005). Modulation of neuronal protein trafficking and function by palmitoylation. *Current Opinion in Neurobiology*, 15(5), 527-535.
- Jiang, J., Ballinger, C.A., Wu, Y., Dai, Q., Cyr, D.M., Hohfeld, J., Patterson, C. (2001). CHIP is a U-box dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. *J Biol Chem* 276:42938–42944.
- Kaltenbach, L. S., Romero, E., Becklin, R. R., Chettier, R., Bell, R., Phansalkar, A., Strand, A., Torcassi, C., Savage, J., Hurlburt, A., Cha, G., Ukani, L., Zhen, Y., Sahasrabudhe, S., Olson, J., Kurschner, C., Ellerby, L., Peltier, J., Botas, J., Hughes, R. E. (2007). Huntingtin Interacting Proteins Are Genetic Modifiers of Neurodegeneration. *PLoS Genetics*, 3(5).
- Kampinga, H.H. and Craig, E.A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nature Reviews. Molecular Cell Biology* 11(8), pp. 579–592.
- Katz, B., & Miledi, R. (1969). Spontaneous and evoked activity of motor nerve endings in calcium Ringer. *The Journal of Physiology*, 203(3), 689-706.
- Kiang, J. (1998). Heat Shock Protein 70 kDa Molecular Biology, Biochemistry, and Physiology. *Pharmacology & Therapeutics*, 80(2), 183-201.

- Kohan, S.A., Pescatori, M., Brecha, N.C., Mastrogiacomo, A., Umbach, J.A. and Gundersen, C.B. (1995). Cysteine string protein immunoreactivity in the nervous system and adrenal gland of rat. *The Journal of Neuroscience* 15(9), pp. 6230–6238.
- George, J. M. (2002). The synucleins. *Genome Biology*, 3(1).
- Gundersen, C.B., Mastrogiacomo, A., Faull, K. and Umbach, J.A. (1994). Extensive lipidation of a Torpedo cysteine string protein. *The Journal of Biological Chemistry* 269(30), pp. 19197–19199.
- Gundersen, C.B. (1995). Cysteine string protein immunoreactivity in the nervous system and adrenal gland of rat. *J Neurosci* 15:6230–6238.
- Greaves, J., & Chamberlain, L. H. (2006). Dual Role of the Cysteine-String Domain in Membrane Binding and Palmitoylation-dependent Sorting of the Molecular Chaperone Cysteine-String Protein. *Molecular Biology of the Cell*, 17(11), 4748-4759.
- Greaves, J., Lemonidis, K., Gorleku, O. A., Cruchaga, C., Grefen, C., & Chamberlain, L. H. (2012). Palmitoylation-induced Aggregation of Cysteine-string Protein Mutants That Cause Neuronal Ceroid Lipofuscinosis. *Journal of Biological Chemistry*, 287(44), 37330-37339.
- Leveque, C., Pupier, S., Marqueze, B., Geslin, L., Kataoka, M., Takahashi, M., De Waard, M., Seagar, M. (1998). Interaction of cysteine string proteins with the alpha1A subunit of the P/Q-type calcium channel. *J Biol Chem* 273:13488–13492.
- Levine, M.S., Cepeda, C., Hickey, M.A., Fleming, S.M., Chesselet, M.F. (2004). Genetic mouse models of Huntington's and Parkinson's diseases: illuminating but imperfect. *Trends Neurosci* 27:691–697.
- Linder, M.E. and Deschenes, R.J. (2007). Palmitoylation: policing protein stability and traffic. *Nature Reviews. Molecular Cell Biology* 8(1), pp. 74–84.
- Lindquist, S. (1988). The Heat-Shock Proteins. *Annual Review of Genetics*, 22(1), 631-677.
- Mastrogiacomo, A., Gundersen, C.B. (1995). The nucleotide and deduced amino acid sequence of a rat cysteine string protein. *Brain Res Mol Brain Res* 28:12–18.
- Mole, S.E., Williams, R.E. and Goebel, H.H. (2005). Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. *Neurogenetics* 6(3), pp. 107–126.
- Mole, S.E., Williams, R.E. (2010). Neuronal Ceroid Lipofuscinoses. In: Pagon, R.A., Bird, T.D., Dolan, C.R., Stephens, K., eds. *Gene Reviews* [Internet] Seattle (WA): University of Washington, Seattle.
- Nie, Z., Ranjan, R., Wenniger, J.J., Hong, S.N., Bronk, P. and Zinsmaier, K.E. (1999). Overexpression of cysteine-string proteins in *Drosophila* reveals interactions with syntaxin. *The Journal of Neuroscience* 19(23), pp. 10270–10279.

- Nijssen, P.C., Ceuterick, C., van Diggelen, O.P., Elleder, M., Martin, J.J., Teepen, J.L., Tyynelä, J. and Roos, R.A. (2003). Autosomal dominant adult neuronal ceroid lipofuscinosis: a novel form of NCL with granular osmiophilic deposits without palmitoyl protein thioesterase 1 deficiency. *Brain Pathology* 13(4), pp. 574–581.
- Nosková, L., Stránecký, V., Hartmannová, H., Přistoupilová, A., Barešová, V., Ivánek, R., Huřková, H., Jahnová, H., van der Zee, J., Staropoli, J.F., Sims, K.B., Tyynelä, J., Van Broeckhoven, C., Nijssen, P.C., Mole, S.E., Elleder, M. and Kmoch, S. (2011). Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult onset neuronal ceroid lipofuscinosis. *American Journal of Human Genetics* 89(2), pp. 241–252.
- Ohyama T., Verstreken, P., Ly, C.V., Rosenmund, T., Rajan, A., Tien, A.C., et al. (2007). Huntingtin-interacting protein 14, a palmitoyl transferase required for exocytosis and targeting of CSP to synaptic vesicles. *J. Cell Biol.* 179, 1481–1496.
- Ranjan, R., Bronk, P., Zinsmaier, K.E. (1998). Cysteine string protein is required for calcium secretion coupling of evoked neurotransmission in *Drosophila* but not for vesicle recycling. *J Neurosci* 18:956–964.
- Roote, J., and Prokop, A. (2013). How to design a genetic mating scheme: a basic training package for *Drosophila* genetics. *G3 (Bethesda)* 3, 353-358.
- Rozas, J.L., Gómez-Sánchez, L., Mircheski, J., Linares-Clemente, P., Nieto-González, J.L., Vázquez, M.E., Luján, R. and Fernández-Chacón, R. (2012). Motorneurons require cysteine string protein- $\alpha$  to maintain the readily releasable vesicular pool and synaptic vesicle recycling. *Neuron* 74(1), pp. 151–165.
- Royle, S. J. (2006). The cellular functions of clathrin. *Cellular and Molecular Life Sciences*, 63(16), 1823-1832.
- Ruiz, R., Biea, I., & Tabares, L. (2014).  $\alpha$ -Synuclein A30P decreases neurodegeneration and increases synaptic vesicle release probability in CSP $\alpha$ -null mice. *Neuropharmacology*, 76, 106-117.
- Sadzot, B., Reznik, M., Arrese-Estrada, J., & Franck, G. (2000). Familial Kufs' disease presenting as a progressive myoclonic epilepsy. *Journal of Neurology*, 247(6), 447-454.
- Schmitz, F., Tabares, L., Khimich, D., Strenzke, N., de la Villa-Polo, P., Castellano-Muñoz, M., Bulankina, A., Moser, T., Fernández-Chacón, R. and Südhof, T.C. (2006). CSP $\alpha$  deficiency causes massive and rapid photoreceptor degeneration. *Proceedings of the National Academy of Sciences of the United States of America* 103(8), pp. 2926–2931.
- Schultz, M. L., Tecedor, L., Chang, M., & Davidson, B. L. (2011). Clarifying lysosomal storage diseases. *Trends in Neurosciences*, 34(8), 401-410.
- Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H., Rothman, J. E. (1993a). A protein assembly-disassembly pathway *in vitro* that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* 75, 409–418.

- Sharma, M., Burré, J., Bronk, P., Zhang, Y., Xu, W. and Südhof, T.C. (2012). CSP $\alpha$  knockout causes neurodegeneration by impairing SNAP-25 function. *The EMBO Journal* 31(4), pp. 829–841.
- Silver, P.A., Way, J.C. (1993). Eukaryotic DnaJ homologs and the specificity of Hsp70 activity. *Cell* 74, 5–6.
- Simonati, A., Pezzini, F., Moro, F., & Santorelli, F. (2014). Neuronal Ceroid Lipofuscinosis: The Increasing Spectrum of an Old Disease. *Current Molecular Medicine*, 14(8), 1043-1051.
- Sweitzer, S. M., & Hinshaw, J. E. (1998). Dynamin Undergoes a GTP-Dependent Conformational Change Causing Vesiculation. *Cell*, 93(6), 1021-1029.
- Tiwari, S. S., D'Orange, M., Troakes, C., Shurovi, B. N., Engmann, O., Noble, W., Hortobagyi, T., Giese, K. P. (2015). Evidence that the presynaptic vesicle protein CSP $\alpha$  is a key player in synaptic degeneration and protection in Alzheimer's disease. *Molecular Brain*, 8(1), 6.
- Tobaben, S., Varoqueaux, F., Brose, N., Stahl, B., Meyer, G. (2003). A brain-specific isoform of small glutamine-rich tetratricopeptide repeat-containing protein binds to Hsc70 and the cysteine string protein. *J Biol Chem* 278:38376–38383
- Umbach, J.A., Zinsmaier, K.E., Eberle, K.K., Buchner, E., Benzer, S. and Gundersen, C.B. (1994). Presynaptic dysfunction in *Drosophila* csp mutants. *Neuron* 13(4), pp. 899–907.
- Umbach, J.A., Gundersen, C.B. (1997). Evidence that cysteine string proteins regulate an early step in the Ca<sup>2+</sup>-dependent secretion of neurotransmitter at *Drosophila* neuromuscular junctions. *J Neurosci* 17:7203–7209.
- Umbach, J.A., Saitoe, M., Kidokoro, Y., Gundersen, C.B. (1998). Attenuated influx of calcium ions at nerve endings of csp and shibire mutant *Drosophila*. *J Neurosci* 18:3233–3240.
- Velinov, M., Dolzhanskaya, N., Gonzalez, M., Powell, E., Konidari, I., Hulme, W., Staropoli, J. F., Xin, W., Wen, G. Y., Barone, R., Coppel, S. H., Sims, K., Brown, W. T., Züchner, S. (2012). Mutations in the Gene DNAJC5 Cause Autosomal Dominant Kufs Disease in a Proportion of Cases: Study of the Parry Family and 8 Other Families. *PLoS ONE*, 7(1).
- Vesa, J., Hellsten, E., Verkruyse, L.A., Camp, L.A., Rapola, J., Santavuori, P., Hofmann, S.L. and Peltonen, L. (1995). Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature* 376(6541), pp. 584–587.
- Warrier, V., Vieira, M., Mole, S. E. (2013). Genetic basis and phenotypic correlations of the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta*; 1832(11):1827–30.
- Williams, R. E., & Mole, S. E. (2012). New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology*, 79(2), 183-191.
- Wu, M. N., Fergestad, T., Lloyd, T. E., He, Y., Broadie, K., & Bellen, H. J. (1999). Syntaxin 1A Interacts with Multiple Exocytic Proteins to Regulate Neurotransmitter Release In Vivo. *Neuron*, 23(3), 593-605.

- Zhang, Y.Q. and Chandra, S.S. (2014). Oligomerization of Cysteine String Protein alpha mutants causing adult neuronal ceroid lipofuscinosis. *Biochimica et Biophysica Acta* 1842(11), pp. 2136– 2146.
- Zhao, X., Braun, A.P., Braun, J.E. (2008). Biological roles of neural J proteins. *Cell Mol Life Sci* 65:2385–2396.
- Zinsmaier, K.E., Eberle, K.K., Buchner, E., Walter, N. and Benzer, S. (1994). Paralysis and early death in cysteine string protein mutants of *Drosophila*. *Science (New York)* 263(5149), pp. 977– 980.
- Zinsmaier, K.E. and Imad, M. (2011). Cysteine-String Protein's Role at Synapses. In: Wytenbach, A. and O'Connor, V. eds. *Folding for the Synapse*. Boston, MA: Springer US, pp. 145–176.