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# Investigating the Pathogenecity of CHL1 Leu17Phe Polymorphism in Schizophrenia

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# INVESTIGATING THE PATHOGENECITY OF CHL1 LEU17PHE POLYMORPHISM IN SCHIZOPHRENIA

**by**

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**Approved:**

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**Abstract**

Schizophrenia is a devastating brain disorder that affects a surprising 1% of the world's population. Despite this prevalence, little is known about the molecular aspects of this disorder making it both difficult to diagnose and treat. Several studies have identified the CHL1 gene (Close Homolog of L1), sometimes referred to as CALL, as a risk gene for schizophrenia. CHL1, a neural cell adhesion molecule, has major roles in cell migration, and the development of dendritic and axonal projections. Therefore any deficiency in CHL1 may result in brain defects similar to those identified in schizophrenic populations. Moreover, in genetically engineered mice, studies have shown that deficiency of the CHL1 gene results in altered emotional reactivity (such as altered fear responses) and motor coordination, reduced sensorimotor gating and impaired working memory and spatial-temporal integration, similar characteristics to those seen in patients with schizophrenia.

The focus of this study is to assess the research literature available for CHL1 as well as investigate a single point mutation in the CHL1 sequence altering a leucine residue to a phenylalanine in the signal peptide of the protein (Leu17Phe) in order to produce a functional deficit of the CHL1 gene. This missense polymorphism has been identified as a risk factor for schizophrenia in Asian populations. Through this mutagenesis, we are able to study CHL1 protein recruitment to the cell membrane in order to understand CHL1's role in schizophrenia at a molecular level.

**Acknowledgements**

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## Introduction

### *Cell Adhesion Molecules*

Cell adhesion molecules (CAM) perform a vital role in cell to cell recognition particularly in the development and construction of the nervous system. These proteins are recruited to the surface of the cell membrane where they are capable of interacting with other proteins on other cells as well as the extracellular matrix (ECM) allowing the cells to form viable tissues and detect their surroundings. Research has shown that cell adhesion molecules function not only in the structural capabilities of the cell, via their interactions with the actin cytoskeleton, but are also involved in transduction events (Maness and Schachner, 2007). CAMs contribute to transduction events either directly, through the elicitation of signals after becoming engaged by a previous signal, or through a collaborative way by which they modulate the efficiency of the signal pathway. In this way, CAMs coordinate and regulate the structure and signaling functions of the cell.

CAMs are essential to the developing nervous system. They are prevalently expressed on growing axons and growth cones as well as their targets thereby dictating the movement of axons and the connections they make. This influences the overall structure of the nervous system. The use of CAMs has even been implicated in gene and cell therapy as means to repair damages incurred by neurodegenerative diseases and trauma (Lavdas et al., 2011)

CAMs can be subdivided into five main classes: cadherins, selectins, mucins, integrins, and the immunoglobulin (Ig) superfamily.

*Immunoglobulin Superfamily*

The immunoglobulin superfamily of CAMs (IgCAM) are calcium-independent transmembrane glycoproteins that include an extracellular immunoglobulin-like domain. There are over 765 members in the immunoglobulin superfamily making it one of the most diverse families of proteins in the body (Soroka et al., 2008). This superfamily is categorized by its typical structure consisting of an extracellular domain containing several immunoglobulin-like intra-chain disulfide bonded loops with conserved cysteine residues, a transmembrane domain, and an intracellular domain that functions to interact with the cell's cytoskeleton. This superfamily plays critical roles in synaptic specificity through the use of hetero- and homophilic interactions with proteins on other cells as well as roles in axon growth and axon guidance towards target regions (Furley et al., 1990; Walsh and Doherty, 1997). IgCAMs have been shown to be expressed in developing mice in sensory and motor neurons within the dorsal root ganglion as well as the spinal cord further implicating these molecules have a significant role in the developing nervous systems (Gu et al., 2015).

*L1 CAM Family*

Within the immunoglobulin superfamily there is a subset of CAMs known as the L1 family. These proteins are evolutionarily well-conserved, with a structure consisting of 13 distinct protein domains, typically with six immunoglobulin and five fibronectin type III domains. The six immunoglobulin-like domains occur at the end of the extracellular region of these proteins and each protein has a single pass transmembrane region as well as a short cytoplasmic tail. It was originally thought that the immunoglobulin

domains of L1-type proteins belonged in the C2 set of immunoglobulin proteins; however, comparison with other immunoglobulin type proteins revealed that these domains are actually novel to the L1-type proteins and therefore should be reclassified into the I set (Bateman et al., 1996; Harpaz and Chothia, 1994). The second immunoglobulin domain is involved in the homophilic adhesive interactions of L1-type proteins (Hortsch et al., 2009; Maness and Schachner, 2007).

The cytoplasmic domains of the L1-type protein range from 85 to 148 residues and contain several conserved motifs that include the amino acid tyrosine. The ankyrin-binding site of L1-type proteins consists of one of these important motifs: FIGQY (Garver et al., 1997). When FIGQY is phosphorylated, ankyrin can no longer bind to the L1-type protein (Garver et al., 1997; Whittard et al., 2006). This interaction with ankyrin is believed to stabilize the axonal membranes and or the intercellular connections (Buhusi et al., 2003). The homophilic L1-L1 interaction does not require the whole cytoplasmic domain; however, both intracellular and extracellular interactions are needed to influence and regulate the various functions of L1 including Ankyrin binding (Guan and Maness, 2010; Hortsch et al., 1998; Hortsch et al., 2009; Ooashi and Kamiguchi, 2009; Wong et al., 1995).

The L1 family contains at least four known proteins: L1-CAM, neurofascin, NrCAM (neuron-glia-related cell adhesion molecule), and CHL1 (Close Homolog of L1). It is believed that these four paralogs arose due to two gene duplication events that occurred genome wide in early chordate evolution (Kappen et al., 1989; Mualla et al., 2013; Schughart et al., 1989). An additional two genes exist per each L1 paralog

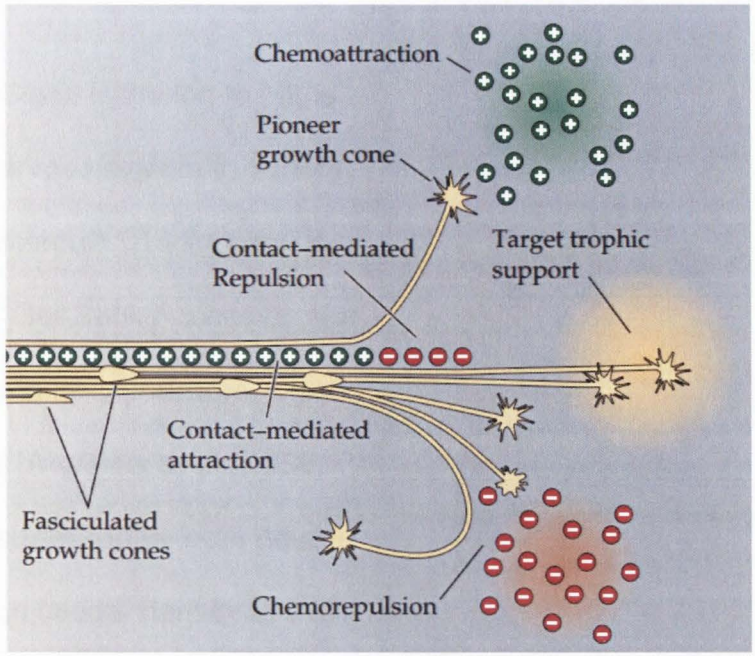
among vertebrates due to a third duplication event that occurred in the teleost lineage resulting in a total of eight L1-type genes in each genome (Mualla et al., 2013).

The vast majority of L1-type proteins are expressed in the nervous system; however, they are also found in many other types of tissues. These proteins undergo both hetero- and homophilic interactions and are capable of acting as both their own ligand and receptor. Some L1-type proteins function in fasciculation, or bundling of groups of axons (Schmid and Maness, 2008). All proteins within the L1 family are important in the guidance and growth of neurons during the development of the nervous system.

*Axonal Growth and Guidance*

The correct synaptic connections made during development are of the upmost importance for an appropriately functioning nervous system. The disruption of axon guidance in both the central and peripheral nervous system during human development has been linked to many serious brain disorders including sensory and motor deficits as well as intellectual deficiencies and cognitive and social impairments (Irintchev et al., 2004). Axon guidance relies on both chemoattraction and chemorepulsion. Developing axons form growth cones with CAMs on their surfaces. The CAMs are able to respond by growing towards target-derived signals and away from chemorepellent regions (Figure 1). A primary repulsive cue are the semaphorin proteins. Semaphorins interact with plexins and neuropilins on the growth cones of developing neurons causing these growth cones to collapse thereby ceasing axon extension (Figure 1).

The L1-type protein CHL1 has been shown to interact with ezrin-radixin-moesin (ERM) in order to mediate Sema3A-induced growth cone collapse (Schlatter et al., 2008). The RGGKYSV sequence in the juxtamembrane portion of the cytoplasmic domain of CHL1 recruits ERM to the plasma membrane. The CHL1/ERM complex interacts with neuropilin-1 and may alter cytoskeletal arrangements, thereby facilitating growth cone collapse (Wright et al., 2007).



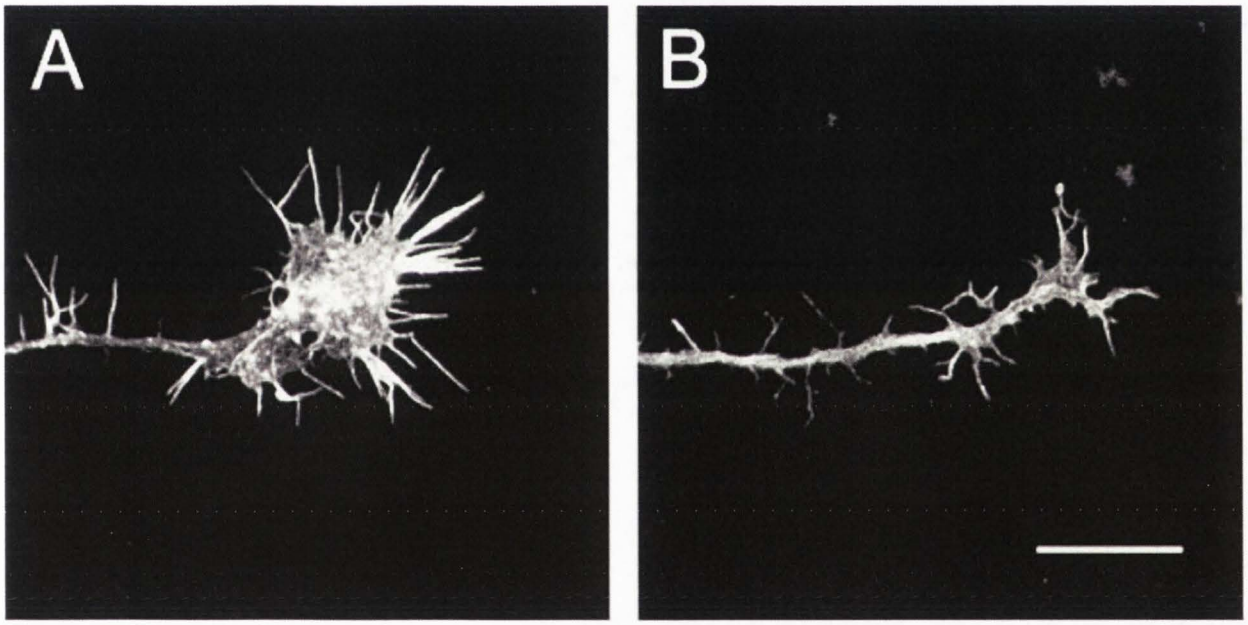




Figure 1. (Top) Mechanism of axonal guidance through chemorepulsion and chemoattraction. (Bottom) A. Growth cone of axon without the presence of Sema3A. B. Growth cone collapse of axon in the presence of Sema3A (pictures from (Purves et al., 2012; Schmid and Maness, 2008)).

**CHL1**

CHL1, or Close Homolog to L1, is a cell adhesion molecule expressed throughout the nervous system in vertebrates. The human ortholog is known as CALL (Cell Adhesion Molecule L1-Like) and is encoded by a gene present on chromosome 3 at position 3p26 (GenBank Accession Number AC011609). Mutations within CHL1 have been linked to 3p- syndrome, which is characterized by mental retardation, as well as schizophrenia (Angeloni et al., 1999; Pratte et al., 2003).

CHL1 is expressed by both neurons and glial cells and has a graded expression pattern with a high caudal density to a low rostral expression in the deep layer neurons of the neocortex in developing mice (Ye et al., 2008). This is in contrast to the L1 protein which is expressed uniformly in the central nervous system and only in neurons (Montag-Sallaz et al., 2002). This supports the notion that although CHL1 is structured similarly to L1, it has a different function. In rodents, CHL1 is expressed late during development around the onset of axogenesis by neurons (Holm et al., 1996). CHL1 is also needed for cortical neuron migration. It is believed that CHL1 regulates area-specific neuronal connections in areas such as the visual and somatosensory cortex (Demyanenko et al., 2004). Impairments in the neuronal connections within these areas could lead to hallucinations as is commonly seen in those diagnosed with schizophrenia.

*Structure*

CHL1 consists of 26 exons over an approximate length of 90 kb (Sakurai et al., 2002). The basic structure of the CHL1 protein includes an N-terminal sequence, six immunoglobin-like domains, 4.5 fibronectin (FN) III repeats, a transmembrane domain, and a C-terminus (Figure 2). The last portion of the FN repeats is rudimentary, consisting of only half of the sequence. It is hypothesized that this half repeat results from alternative splicing (Holm et al., 1996).

CHL1 also contains an RGD sequence (amino acids 185-187) within its second Ig domain on beta strand E. It is thought that this sequence is essential for cell attachment and integrin binding of CHL1 (Holm et al., 1996). Another sequence found in CHL1 is the DGEA sequence (amino acids 555-558). This motif is found on beta strand C on the sixth Ig domain and is not present in other members of the L1 family. This motif functions in $\alpha_2$ $\beta_1$ integrin recognition of type I collagen.

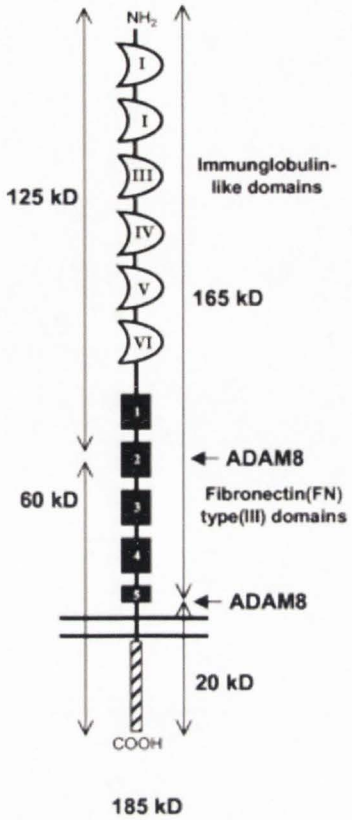



Figure 2. Domain structures of CHL1 overview (picture courtesy of (Naus et al., 2004)).

*Signal Peptide*

The N-terminus of CHL1 contains a signal peptide region. This sequence is a short peptide that mediates the targeting and translocation of the protein to the endoplasmic reticulum membrane. This signal is particularly important for transmembrane proteins and secreted proteins. The signal peptide is vital for the appropriate trafficking of proteins to the correct regions of the cell.

When the nascent polypeptide emerges from the ribosome, the signal sequence at the N-terminus along with the engaged ribosome, known as the ribosome-nascent complex (RNC), interacts with the signal recognition particle (SRP). When the SRP

binds, translation is slowed in an event known as elongation arrest. The SRP then delivers the RNC to an SRP receptor (SR) near a translocon at the target membrane where the RNC is docked. The RNC is then transferred to the translocon machinery and is integrated into the lipid bilayer, or, in the case of secretory proteins, is translocated across the membrane to enter the secretory pathway (Akopian et al., 2013).

This pathway requires energy which is provided by either GTPase or ATPase modules that are located within the targeting machinery. The signal peptides tend to have three regions: a positively charged N-region, a hydrophobic H-region, and a C-region which contains polar amino acids. The co-translational trafficking of these proteins minimizes the misfolding and/or aggregation of nascent proteins before they arrive at their destinations. This is highly advantageous for the targeted delivery of membrane bound proteins and secretory proteins.

The timely recognition of signal peptides by the SRP is crucial for the accurate initiation of co-translational protein targeting. Amino acid substitutions within the three regions of the signal sequence could potentially result in slower recognition of the signal peptide by the SRP ultimately leading to the assortment of these proteins to the wrong cellular locations. When proteins are not expressed in their appropriate locations, their associated functions may not be properly carried out, which could lead to disease. In fact, many studies have shown that mutations within the signal peptide region are correlated to disease (Crockett et al., 2011; Huang et al., 2010; Hussain et al., 2013; Liu et al., 2012; Mencarelli et al., 2012; Qin et al., 2012; Rane et al., 2010). Therefore, mutations within the signal peptide region of CHL1 could potentially lead to disease.

## CHL1 and schizophrenia

Deficiencies and mutations within the CHL1 gene have been associated with schizophrenia. Since the CHL1 gene is highly conserved between species, studies directed at understanding the relationship between CHL1 and schizophrenia were mostly performed in mice. These studies have shown that mice deficient in CHL1 have abnormalities in both brain morphology and behavior that mimic those of schizophrenic patients.

In a study done in 2002, 24 Japanese schizophrenic patients were screened for mutations in the CHL1 gene to identify schizophrenia-risk associated alleles. A missense polymorphism was identified within the signal peptide region of CHL1 that had a significantly higher frequency in the schizophrenic population when compared to the general population. This polymorphism is a mutation of the amino acid leucine to a phenylalanine at position 17 (Sakurai et al., 2002). Further genome-wide studies have also marked this polymorphism as a risk factor in schizophrenia (Chen et al., 2005; Shaltout et al., 2013). The purpose of this study is to investigate the pathogenicity of this point mutation and how it may function in contributing to schizophrenia.

### *Brain Morphology Differences in CHL1 Deficient Mice*

Knocking out the CHL1 gene in mice leads to some noticeable alterations in brain structure when compared to mice with normally functioning CHL1 genes. There are two major regions that show abnormal neuron organization when the CHL1 gene is knocked out: the hippocampus and the thalamocortex. The formation of the hippocampus plays a vital role in sensorimotor processes and is therefore an area of

interest when investigating the neuropathology of schizophrenia (Morellini et al., 2007). Studies have detailed an alteration in hippocampal mossy fiber organization in CHL1 deficient mice irrespective of their background strain (Buhusi et al., 2003; Montag-Sallaz et al., 2002). Overall, there are many organizational changes in brain morphology when comparing CHL1 deficient mice to their wild type littermates. Some of the axons in the CHL1 deficient mice formed either small bundles or moved as individual thin mossy fibers through the CA3 region of the brain thereby forming a network between the suprapyramidal and infrapyramidal bundles. Many synaptic boutons surrounding the pyramidal cell soma were also observed (Montag-Sallaz et al., 2002). This indicates that terminals were formed on the cell bodies, not just the proximal part of the pyramidal cell dendrites.

One study performed on 3 week old CHL1 knockout mice showed an increase in the inhibitory postsynaptic currents evoked in pyramidal cells through minimal stimulation of perisomatically projecting interneurons. This study also observed that mice deficient in CHL1 demonstrated age-dependent deficits in long term potentiation and perisomatic inhibition. These deficits became more pronounced with age (Dityatev et al., 2008). Projections to the dentate gyrus have been found to be enhanced in CHL1 deficient mice (Morellini et al., 2007). These enhanced projections may demonstrate the correlation between the altered behavioral response to environmental and social stimuli of CHL1 knockout mice and the alterations in their brains.

CHL1 deficient mice also had alterations in their olfactory axon projections and thalamo-cortical neuroanatomy (Demyanenko et al., 2004). In addition, there is also a displacement of pyramidal neurons in layer V of the visual cortex (Demyanenko et al.,

2004). Further observations of aberrant neuron migration in the olfactory bulbs of CHL1 deficient mice show that some of the axons either cross the glomerular layer without branching and terminate in the external plexiform layer, the underlying bulbar layer, or connect to more than one glomerulus (Montag-Sallaz et al., 2002).

The temporary absence of CHL1 results in some axonal ensembles and axons being incapable of finding the appropriate targets and the permanent absence of CHL1 is linked to misguided axonal projections and aberrant axonal connectivity (Montag-Sallaz et al., 2002). These findings support the notion of the involvement of CHL1 in psychiatric disorders such as schizophrenia as many of these abnormal brain structures are responsible for behaviors, such as sensory gating, that is impaired in schizophrenic patients (Braff et al., 2001; Irintchev et al., 2004).

*Behavioral Differences in CHL1 Deficient Mice*

Mice deficient in CHL1 have many abnormal behaviors when compared to wild type mice. The CHL1 deficient mice react differently to open field tests, elevated plus maze tests, and Morris water maze tests. The deletion of the CHL1 gene results in changes to other behavioral parameters such as motor coordination, delayed responsiveness to environmental stimuli, exploration, and stress alterations (Morellini et al., 2007; Pratte et al., 2003). In the open field test, CHL1 deficient mice spent more time in the central area of the field as compared to their wild type littermates who tended to avoid the center of the field. This may indicate a different exploratory behavior of reduced anxiety. It is also speculated that CHL1 deficient mice simply failed to notice the novelty of their environment in these tests and therefore explored all parts of the

field equally which would explain their reduced anxiety (Pratte et al., 2003). Knockout mice demonstrated milder reactions to stress inducing tests as well when compared to wild type mice, this behavior is commonly associated with the reduced anxiety in the open field test as shown in prior studies (Montag-Sallaz et al., 2002; Pratte et al., 2003). CHL1 deficient mice also showed significant differences in terms of path swimming during the Morris water maze. CHL1 deficient mice had similar swim patterns when compared to their wild type littermates, this suggests that spatial memory is not impaired by CHL1 deficiency. However, on the first day of reversal, the mutants showed more crossing over the old goal, or perseveration, a trait commonly seen in schizophrenia that may relate to altered plasticity (Montag-Sallaz et al., 2002).

Another study observed that CHL1 deficient mice are impaired in prepulse inhibition which measures the ability of the central nervous system to gate the flow of sensory information (Dityatev et al., 2008). This impaired sensory-motor gating is often seen in schizophrenic patients (Braff et al., 2001; Irintchev et al., 2004). This study also implies that if CHL1 is deficient at the intermediate stages of development, the deficit is compensated for; however, it appears later as impaired long term potentiation during the early and late stages of postnatal development (Dityatev et al., 2008).

CHL1 knockout mice have deficits in interval timing (Buhusi et al., 2003). Interval timing is necessary for decision-making and motor control. Impairments in interval timing have been observed in neuropsychiatric disorders, such as schizophrenia. CHL1 deficient mice show a maintained leftward shift in the response curve when compared to the responses of their wild type littermates. This means that the maximum response rate for CHL1 deficient mice during a timing task occurred earlier than the peak

response rate for wild type mice. This indicates a deficit in their memory encoding and decoding capabilities (Buhusi et al., 2003). In a spatial-temporal task, CHL1 deficient mice made more errors in working and reference memory than the controls. Timing impairments such as these have been reported in schizophrenia in numerous studies (Braus, 2002; Green and Nuechterlein, 1999; Herzog and Brand, 2009; Kimura, 2003; McDowell et al., 1996; Volz et al., 2001). These impairments may be due to the alterations in hippocampal circuitry.

The purpose of this study was to assess the point mutation Leu17Phe in the signal peptide region of CHL1 and determine the underlying mechanisms of pathogenecity that may contribute to the link between this mutation and schizophrenia.

**Methods**

In order to study the function of the point mutation resulting in the amino acid change of leucine to phenylalanine within the signal peptide region of CHL1, we have decided to investigate the protein trafficking of CHL1 on the cellular level. Studies investigating this mutation's effect on CHL1 protein trafficking have not been conducted before and we hypothesize that this mutation in the signal peptide of CHL1 will alter the protein's recruitment to the membrane thereby leading to the abnormal brain development seen in the schizophrenic patients.

For this project, a plasmid called pcDNA3 with the wild type copy of the CHL1 gene already inserted into the multiple cloning site (MCS) at the EcoRI restriction site was used. This plasmid was transformed into XL10 Gold *E. coli* cells in order to

produce a large stock of the plasmid. The plasmids were then isolated from the cells using a Qiagen Miniprep Kit.

Site-directed mutagenesis was performed using polymerase chain reaction (PCR) with primers designed as per manufacturer protocol for the QuickChange II XL Mutagenesis Agilent Kit and based on the CHL1 gene sequence found at NCBI in accession number AAHY01055945 (http://www.ncbi.nlm.nih.gov). These primers contain the single base change needed to alter the codon for leucine (CTC) to phenylalanine (TTC) within the CHL1 signal peptide region (Table 1).

Table 1. Sequences of Mutagenesis Primers

| Primer | Sequence |
|---|---|
| Forward | 5'-tgctgacaattttaacaggaagaaaattagacttaggatcagtcc-3' |
| Reverse | 5'-ggactgatcctaagtctaattttcttcctgttaaaattgtcagca-3' |

After the PCR reaction, the new copies of the DNA should contain the mutation present in the primers. The linear PCR product is used to transform XL10 Gold bacteria, which allows for the re-circularizing of the DNA obtained from the PCR. The bacteria were selected on Ampicillin LB Agar plates, plasmid DNA from selected clones was purified with Qiagen Miniprep kits and then sent to a commercial sequencing agency in order to evaluate the presence of the mutation as well as the accuracy of the rest of the sequence. Unfortunately, due to unforeseeable circumstances (plasmid DNA degraded due to Freezer failure) the mutation was not obtained. The rest of the project has not been performed at this time, but will continue in the methods outlined

below.

If the mutation occurs and the sequence is correct, both the wild type form of CHL1 and the mutated CHL1 gene (Leu17Phe) in the plasmid will undergo an additional PCR step for the amplification of the gene. In this PCR reaction, we will only be amplifying the CHL1 cDNA (wild type or mutated depending on the plasmid). The appropriate restriction sites will be added to the gene with the promoter at this time as well. The CHL1 sequence will be amplified without the stop codon. The PCR product will then be purified. The wild type and the mutated copies of CHL1 will then be digested, or spliced, at the specific sites that were determined by our primer selection.

For this project, an expression vector known as pTimer1 will be used. This vector contains a specific gene for a modified green fluorescent protein (GFP). When a gene of interest is ligated into the pTimer1 plasmid, it is inserted so that the genes fuse together. This way the expressed CHL1 protein will contain the modified GFP at the C-terminal end. The modified GFP, in this case DsRed1-E5, allows for easier observation of CHL1 protein trafficking via microscopy. DsRed1-E5 is known as a modified GFP since when the protein is first expressed, it fluoresces in the green spectrum of light; however, as the protein ages, a red fluorophore emerges instead. This color shift from green to red is therefore useful in monitoring protein expression at the cell membrane as well as the rate of recycling and trafficking of new CHL1 protein to the membrane.

At this time in the experiment, we will also digest the pTimer1 plasmid with the same enzymes used to digest the CHL1 mutant and wild type plasmids. The wild type gene can now be ligated into the digested pTimer1 plasmid and the mutated gene can

be ligated into another digested pTimer1 plasmid (a general outline of these steps can be found in figure 3.). These reactions will be put into XL10 Gold bacteria again with a selective antibiotic in the plated LB agar in order to obtain recombinant clones. The bacteria that do not take up the plasmid will not be able to survive on these plates. A stock of these plasmids will be produced in XL10 Gold and then the plasmids will be transfected into HEK293 cells along with lipofectamine which will produce a stable cell line. The success of these steps will create a new, workable way to study a novel mutation in the CHL1 gene. Various studies, including CHL1 signaling and protein recruitment differences, can be studied at a smaller level and can then be correlated to the molecular mechanisms that perturb neuronal function leading to schizophrenia.

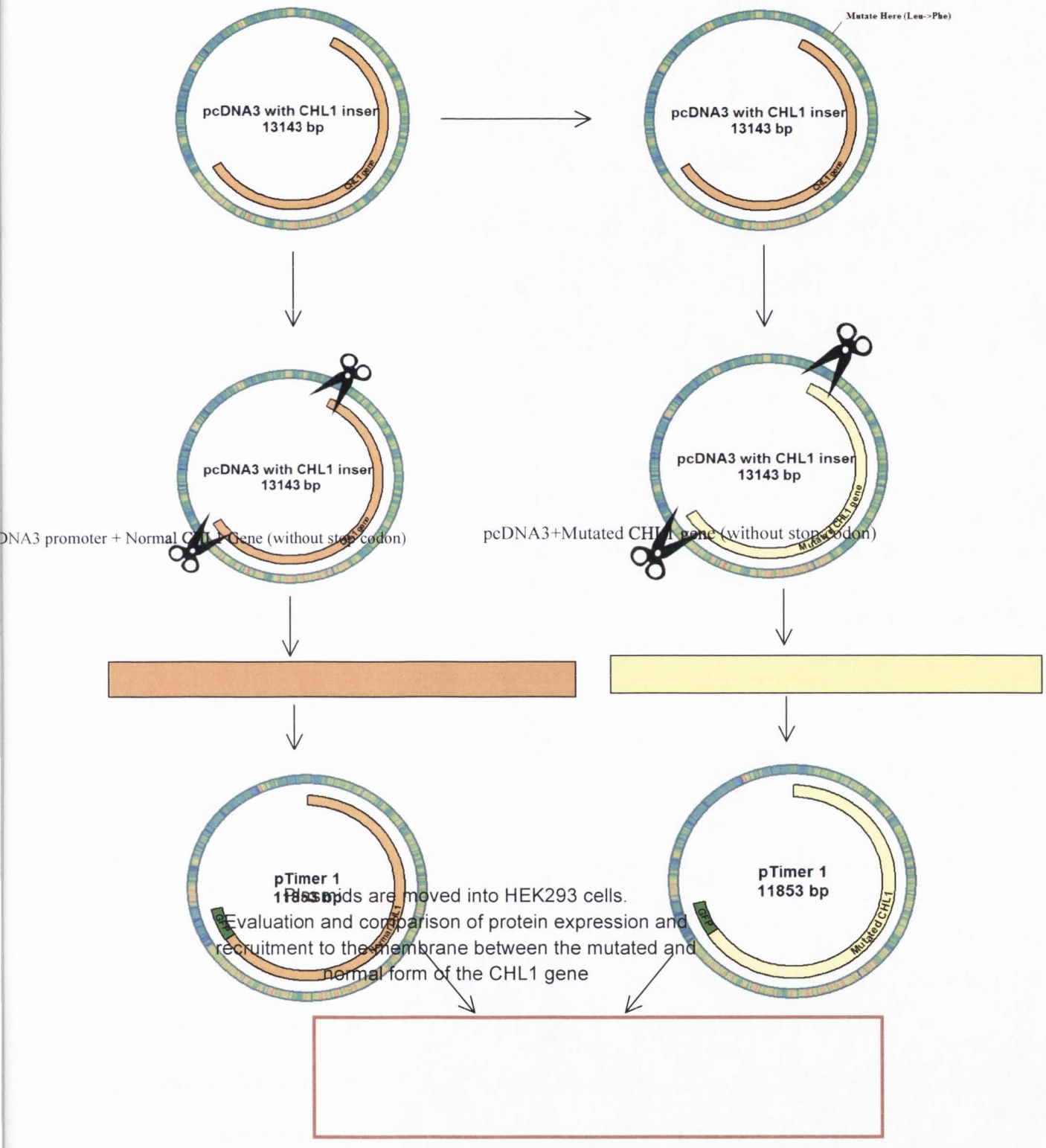



Figure 3. A general overview of the mutation of the CHL1 gene and the movement of the wild type and mutated gene to the pTimer plasmid which allows for the comparison and observation of CHL1 protein expression.

**Expected Results**

We predict that the CHL1 proteins with the mutated signal peptide will be trafficked less efficiently to the cell membrane, if at all, in comparison to the CHL1 proteins without the mutation. Therefore, we would expect the cells expressing the mutated signal sequence (Leu17Phe) in CHL1 to have a membrane that is redder whereas the cells expressing the wild type CHL1 would be greener (Figure 4). This is because the SRP is able to recognize the signal peptide on the CHL1 wild type efficiently and therefore it is moved to the endoplasmic reticulum appropriately. This allows the recycling of the protein at the membrane to occur quickly, therefore the modified GFP is not expressed at the membrane long enough for us to see the shift from green absorption to red. The length of time necessary for the trafficking of CHL1 will be determined by preliminary observation of trafficking in CHL1 WT cells. The GFP begins to turn red 3 hours after initial expression after which the shade of red deepens as the expression time lengthens.

In juxtaposition, the mutated signal peptide should be more difficult for the SRP to recognize or may cause the machinery to sort the proteins incorrectly relocating the protein to another part of the cell. When the SRP cannot recognize the signal peptide, there will be no halt in translation of the mRNA by the ribosome. This may lead to the misfolding of the CHL1 protein which would mark the protein for degradation, further emphasizing the necessity of a quick recognition response of the SRP for the signal peptide. If the SRP fails to recognize the mutant CHL1 signal peptide there is an inhibition of protein recycling at the plasma membrane. Without this recycling, the CHL1 with the modified GFP tag shifts from green to red as the protein ages.

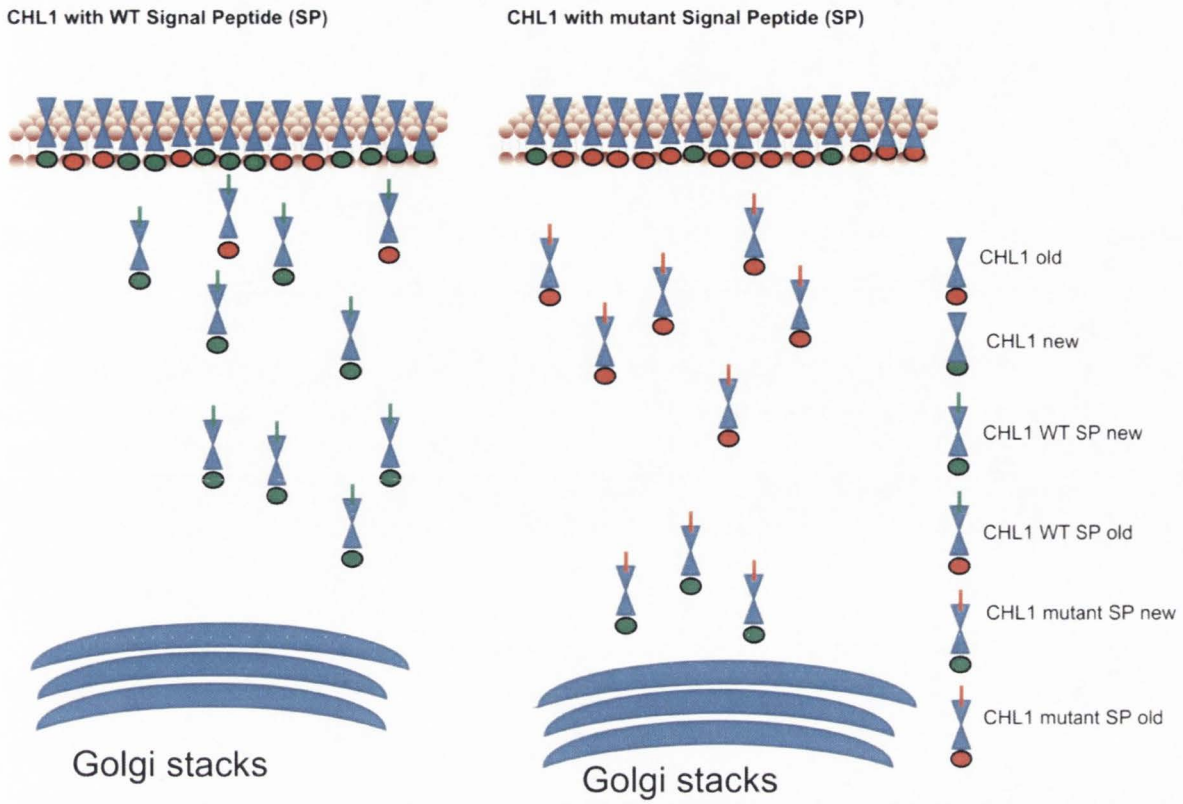



Figure 4. Expected recruitment of tagged CHL1 to the cell membrane for both wild type and mutated CHL1 proteins

## Discussion

Unfortunately, the mutant CHL1 plasmid was not obtained; therefore results are only speculated at this time. According to the literature currently available at this time we believe that a mutation within the signal peptide region of the CHL1 protein would alter the trafficking of this protein. Based on the function of CHL1 in the developing nervous system, we conclude that a disruption of CHL1 trafficking could be an underlying factor in the brain morphology differences and aberrant axonal pathways and behaviors witnessed in patients diagnosed with schizophrenia. Due to this lack of results, however, these conclusions still remain as solely speculations. A new CHL1

wild type plasmid has been obtained in order to repeat procedures in the hopes that the leucine to phenylalanine mutation may be created and studied.

There are a few speculations as to why we did not achieve the CHL1 mutant. A nanodrop test of the sterile water revealed that the molecular grade water had been contaminated and exhibited absorbance patterns similar to protein and nucleoside contamination. Another possible error could be due to a freezer issue experienced prior to the start of the experiment. At that time, the wild type CHL1 plasmid was being stored in the -80°C freezer. The power supply to that freezer failed and the backup generator did not initiate. Therefore the plasmid experienced a drop in temperature for an undetermined period of time, this could have resulted in nicks within the CHL1 plasmid DNA.

In an attempt to fix these nicks, the CHL1 plasmid DNA was moved into XL10-Gold *E. coli*, which are bacteria commonly characterized by their ability to fill in nicked DNA. However, since we still did not obtain the mutant CHL1, it is unsure whether or not the bacteria were actually able to fix the nicked DNA. Gel electrophoresis was ran on the wild type CHL1 plasmid to view whether or not the plasmid DNA was in pieces or cohesive. The gel showed one large band at the appropriate size for the CHL1 wild type plasmid suggesting the DNA is not nicked but may still contain damage that makes it nonfunctional. Therefore, we believe the best measure to take at this time would be to start the project again using a new CHL1 wild type plasmid.

**Conclusion**

CHL1 is a vital protein for the appropriate development of the nervous system; however, this protein remains understudied and its function is not well understood. Therefore, continued research on this protein is essential for understanding the mechanisms of many diseases such as schizophrenia as well as providing more knowledge on how the nervous system develops. Studies previously done have identified CHL1 as an important contributor in many behavioral paradigms (Baddeley and Salame, 1986; Braff et al., 2001; Irintchev et al., 2004; Kolata et al., 2008; Morellini et al., 2007). When the CHL1 gene is knockout, mice exhibit altered emotional reactivity and motor coordination, as well as reduced sensorimotor gating, impaired working and spatial-integration. These abnormal behaviors may be linked to the altered brain morphology in CHL1 knockout mice wherein there are alterations to axonal guidance leading to inappropriate neuronal connections particularly in the hippocampal regions during development (Montag-Sallaz et al., 2002).

Continued attempts to achieve the correct missense polymorphism will be made and the study will be completed at a later time. This research will continue based on whether or not the expected results are achieved. If there is shown to be a significant difference in the recruitment of the mutated CHL1 to the membrane between the mutant and the wild type, then further studies may be done to better understand the underlying mechanism. In particular, a new mouse model could be made that expresses this mutant CHL1 (Leu17Phe). This model could then be used to study the behavioral effects of the point mutation. If the mutant CHL1 is not recruited differently in comparison to the wild type cells, further research would involve choosing another point

mutation to investigate. The study done by Sakurai et al. identified other mutations in the CHL1 gene among the schizophrenic population that could also be studied (Sakurai et al., 2002).

**Reflective Writing**

Overall, I think that my thesis experience was essential to my undergraduate degree. I feel like I have learned the most from my thesis in comparison to the rest of my course material. By doing this thesis project, I was able to apply the knowledge I have learned in my biology and chemistry coursework to a real life research experience. Although the project was a bit frustrating at times, I was able to develop valuable skills in troubleshooting problems that arose throughout the experiment. I have gained a lot of respect and appreciation for researchers and the scientific community in general from doing this project.

There were a few problems faced while working on this thesis. The agar plates after a 24 hour incubation period would be found cracked and dried up. The temperature of the incubator was checked and held steady at 37°C, so it was determined that that was not the issue. After adding some water to the incubator while the plates were incubating, the shrinkage did appear to slow down somewhat but did not stop overall. I also checked to ensure that none of the liquid evaporated from the agar solution during the liquid autoclaving which ultimately result in a higher concentration. The final thought process was to change the agar mixture brand. This did seem to help and we did not have issues with the agar drying out after this, although during this time there was also a lot of rain which did raise the overall humidity in the lab so we cannot determine that this was a complete fix.

Another issue that we faced was being unable to achieve a sequence for the clones sent in for sequencing. There are a couple of factors that could have contributed to this issue. A sample of primer and water was analyzed via nanodrop and was

determined to have been contaminated. This contributed to the larger problem in the sequencing as the clones were diluted using the contaminated molecular grade water and the sequencing was run using the primers made from the contaminated water. Also, many of the clones' DNA were really low in concentration even after utilizing minipreps. In order to resolve this problem, less water was used at the end to elute the DNA which did bring the concentration up slightly, however, did not yield as high of a concentration as expected. A final factor that we are now investigating is a problem with the freezers that occurred before the project started. At that time, the -80°C freezer went down for a while with no power, therefore the stock wild type plasmid that was being stored in this freezer warmed up for an indeterminate amount of time. This may have caused nicking in the DNA that would have deemed the plasmid useless for use in the project. Although we attempted to check that the plasmid was not nicked (using gel electrophoresis) and tried to fix any nicks that did occur (by placing the plasmid in XL10-Gold *E. coli*—bacteria that is known for their ability to re-circularize DNA and fix DNA nicks), the fact that a clone was not achieved means that this could still be a problem. In order to troubleshoot this factor, a new CHL1 wild type plasmid was obtained from another laboratory. Testing on this plasmid has not yet been conducted, ergo, we cannot yet determine if this is the solution or not.

The literature review portion of the thesis was very interesting but also incredibly overwhelming. There is a lot of research being done within the field which makes it difficult to narrow down which articles will be most applicable to this research and will contribute to a cohesive paper. During this research, I was impressed by how many different ways people were researching similar ideas. The amount of methods out there

is astounding. I used to be under the impression that there were not a lot of new things to discover, but in reading these articles, so many researchers are investigating different paths for the same main idea. This has really opened my eyes to all of the possibilities for research out there. A lot of the mechanisms underlying the necessary functions of life are still unknown which makes the prospects of research all the more appealing to me.

Ultimately, this thesis project has been very beneficial to my undergraduate career. I was able to learn how to troubleshoot problems that occur during research and use the resources around me (such as other researchers, mentors, and the internet) to my advantage. I was also able to improve my ability to read and understand scientific literature. I enjoyed being able to use knowledge from my undergraduate courses in order to actually understand how genes and proteins function and how alterations to a gene can lead to drastic changes in the organism. These mutations can alter protein trafficking, gene expression, amino acid association–which ultimately alters protein form and furthermore its function, and many other processes within the cell. Even though I did not receive results for this experiment, I learned a lot from this experiment. I feel like I am now more prepared to handle problems that may arise in a laboratory setting after my experience with this thesis. Being capable of handling these situations is paramount to my career as a scientist and so I believe that I am much better suited for a research environment now then I was when I first started my thesis. I also learned a lot about the CHL1 protein which I thought was a lot of fun to know about in detail and to see it functioning as a specific example of many of the processes that I have learned about throughout my coursework like the use of a signal

peptide sequence in protein trafficking. I definitely benefited from my experience with this project and paper and will be able to draw from my experiences in later years when I am conducting my own research.

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