

# Detection of mutations in *PSEN1* and *PSEN2* genes

Ashlee Junier

Forensic Science, Biology, Biotechnology

Faculty Mentor: Dr. Charles Vigue

## Abstract

The genes *presenilin 1* (*PSEN1*) and *presenilin 2* (*PSEN2*) are responsible for the formation of amyloid proteins in the brain. Mutations in these genes lead to errors in the protein formation, which can ultimately lead to the development of protein aggregates common to Alzheimer's disease. Using the National Center for Biotechnology Information and the Coriell Cell Bank, common missense mutations related to Alzheimer's disease in *PSEN1* and *PSEN2* were identified. *PSEN1* involves a cytosine to adenine substitution resulting in glutamate instead of arginine at codon 246, while *PSEN2* involves an adenine to thymine substitution resulting in isoleucine instead of asparagine at codon 141. Primers were developed for each of these genes so that the areas containing the identified mutations were present. Using the primers, the genes were amplified using the polymerase chain reaction. The resulting DNA copies were then digested using restriction enzymes identified to specifically digest the mutated copy of the DNA while leaving the normal copy uncut. In the case of *PSEN1*, the C to A substitution results in the sequence CTGAG. The restriction endonuclease *DdeI* cuts DNA at this sequence, but not at the normal sequence CTGCG. In the case of *PSEN2*, the A to T substitution results in the sequence GATC. The restriction endonuclease *DPNII* cuts DNA at this sequence, but not at the normal sequence GAAC. The digestion products were electrophoresed on an agarose gel (2% in the case of *PSEN1*, 3.5% in the case of *PSEN2*) to view the resulting products. Both the mutations in *PSEN1* and *PSEN2* were able to be visualized on the gel and the normal DNA could be distinguished from the mutant DNA.

## Introduction

Alzheimer's disease (AD) is the most common form of dementia worldwide<sup>1</sup>, affecting millions of people and their families every year. AD is caused by a buildup of amyloid proteins in the brain, which leads to the destruction of neurons and subsequently a loss of cognition and function. There are different forms of AD, including late-onset AD and early-onset AD. People with early-onset AD generally begin to show symptoms before age 60<sup>1</sup>. While there are many factors that contribute to the development of AD, it has been shown that genetic mutations have a substantial influence on the onset of AD<sup>1</sup>.

Genetic mutations occur at the DNA level, and can either be passed on from parents to children or can arise spontaneously. DNA is made of over three billion base pairs, or nucleotides, which are in the form of adenine, thymine, cytosine, and guanine, or A, T, C, and G, respectively. These base pairs form a complex and specific code that is eventually translated into amino acids, which then, in turn, make up proteins. Proteins carry out all of the functions in the body, from the breakdown of food to energy and producing hormones and other essential chemicals to aiding in healing and assisting with memory.

There are many types of genetic mutations that can occur, but the most common are single base pair mutations. These occur when a single nucleotide is switched with another during DNA replication. Since the precise order of nucleotides codes for a specific sequence of amino acids, one incorrect base pair can mean changes in a protein, which may lead to a protein that does not function properly.

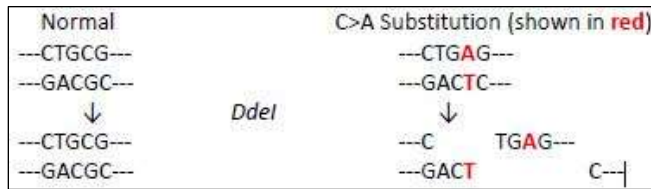
This is the case with the two genes investigated in this study.

The presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes have both been implicated as having a role in early-onset AD. *PSEN1* and *PSEN2*, located at chromosome 14q24.3<sup>2</sup> and 1q42.13<sup>3</sup>, respectively, code for structurally similar proteins that are part of a complex responsible for the cleavage of amyloid  $\beta$  precursor protein and the formation of amyloid  $\beta$ <sup>2</sup>. Mutations in these genes lead to the incorrect formation of the proteins, which cause the onset of AD symptoms. In this study, two common mutations in *PSEN1* and *PSEN2* that have been linked to early-onset AD were investigated.

For *PSEN1*, the mutation examined was at base pair 56,397 where adenine was replaced by cytosine. This mutation leads to the incorporation of the amino acid glutamate instead of alanine, thus altering the proteins structure and function. For *PSEN2*, the replacement of an adenine with a thymine at base pair 15,032 results in the incorporation of the amino acid isoleucine instead of asparagine, altering the function of the resulting protein. This substitution results in the amino acid isoleucine, rather than asparagine, in the amino acid sequence. Both of these mutations have been linked to the development of early-onset AD<sup>1,4,5</sup>.

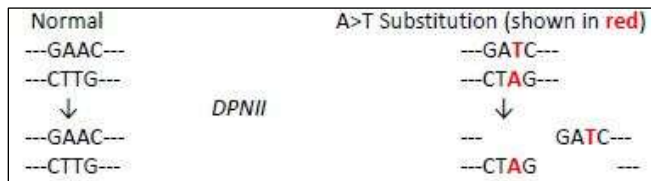
One way to easily detect single base pair mutations is through the use of restriction enzymes. Restriction enzymes are proteins that cut DNA at certain nucleotide sequences. When detecting mutations, an enzyme that recognizes and cuts either the normal or mutated version, but not the other, allows one to distinguish between the two.

For *PSEN1*, the enzyme *DdeI* was chosen because of its ability to recognize and cut the DNA carrying the C>A mutation, but not the normal version. This is illustrated in figure 1.



**Figure 1:** The enzyme *DdeI* does not recognize the sequence CTGCG that is found in the normal DNA (left), leaving it intact. Conversely, *DdeI* does recognize the sequence CTGAG that is found in the mutated DNA (right), cleaving it into the pieces C and TGAG.

In *PSEN2*, the enzyme *DpnII* was chosen because it recognizes the sequence GATC, which is found in the mutated DNA after an A>T substitution. This sequence is not found in the normal DNA, which instead carries the sequence GAAC. This is not recognized by *DpnII*, as can be seen in figure 2.



**Figure 2:** The enzyme *DpnII* does not recognize the sequence GAAC that is found in the normal DNA (left), leaving it intact. Conversely, *DpnII* does recognize the sequence GATC that is found in the mutated DNA (right), cleaving it from the rest of the DNA.

Detection of mutations related to AD is essential in minimizing one's risk of developing the disease. Just as in any disease that carries a variety of risk factors, the key to slowing or stopping AD's development is minimizing risk. If someone knows that they carry a mutation related to early-onset AD in the *PSEN1* or *PSEN2* genes, they can work to decrease their other risk factors by altering their diet, exercise habits, and general lifestyle.

The goal of this study is to be able to distinguish normal DNA from mutated DNA in both *PSEN1* and *PSEN2* using the simple and inexpensive laboratory techniques of PCR, restriction isotyping and gel electrophoresis.

## Materials and methods

First, the exact location of the mutations in *PSEN1* and *PSEN2* were identified. The entire published reference version of each gene was obtained from the National Center for Biotechnology Information (NCBI) and annotated in a word processing program using the information provided by NCBI. Once the location of the mutations was found, primers were developed so that 150-250 bp fragment containing the mutation could be amplified for analysis.

Both the *PSEN1* and *PSEN2* genes were amplified using the polymerase chain reaction (PCR) in 0.2  $\mu$ L Ready-

to-Go PCR tubes from GE Healthcare. The PCR reaction was a total of 25  $\mu$ L, which contained 23  $\mu$ L of dI water, 1  $\mu$ L of primers (5 nm final concentration of each primer), and 1  $\mu$ L of DNA (2 nm final concentration). DNA samples were obtained from the Coriell Cell Repository, which stores cells containing various mutations that can be purchased for use in research. For *PSEN1*, the primer sequences were 5' AAAGGTCCACTTCGACTCCA 3' (L) and 5' GGCATTCTGTGACAAACAA 3' (R). For *PSEN2*, the primer sequences were 5' TCAGCATCTACACGCCATTC 3' (L) and 5' TCTAAAGGCGGCTGTTTCAC 3' (R).

The following combinations of DNA and primers were made to allow for complete analysis of the *PSEN1* and *PSEN2* genes: mutant *PSEN1* DNA with *PSEN1* primers, normal DNA with *PSEN1* primers, mutant *PSEN2* DNA with *PSEN2* primers, and normal DNA with *PSEN2* primers. This ensured that the regions containing the mutations were amplified in both *PSEN1* and *PSEN2*, and also that the same regions were amplified in the normal copies of the DNA to be used for comparison. The reaction containing tubes were placed in the thermocycler for PCR using the following program: 5 minutes at 95°C for denaturation, thirty cycles of 95°C, 56°C, and 72°C for 30 seconds each, and 10 minutes at 72°C for final extension.

PCR of *PSEN1* yielded a 165 bp segment. This was digested with the enzyme *DdeI* from New England BioLabs in a reaction of 7  $\mu$ L dI water, 10  $\mu$ L PCR product, 2  $\mu$ L 10x buffer from New England BioLabs, and 1  $\mu$ L *DdeI* in a 37°C water bath left overnight. Once digestion was complete, tracking dye was added to the samples, which were loaded into a 2% agarose/TAE/ethidium bromide gel. This gel was made by combining 0.8 g of agarose, 40 mL of Tris-EDTA buffer, and 3  $\mu$ L of ethidium bromide. The mixture was heated until boiling, and then allowed to cool until just warm after which 3  $\mu$ L of ethidium bromide was added before being poured into a mold to set. The samples were electrophoresed at 120 volts to allow for the DNA pieces to separate according to size, and then visualized under an ultraviolet light. It was expected that the normal DNA would remain at 165 bp. Because the mutated DNA was heterozygous (the mutation is on one chromosome but not the other), it was expected that some of the DNA would be uncut and remain at 165 bp and some of the DNA would be cut to 89 and 76 bp. The amplified area of *PSEN1* can be seen in figure 3.

normal	AAA GGT CCA CTT CGA CTC CAG CAG GCA TAT
protein	Lys Gly Pro Leu Arg Leu Gln Gln Ala Tyr
normal	CTC ATT ATG ATT AGT GCC CTC ATG GCC CTG GTG
protein	Leu Ile Met Ile Ser Ala Leu Met Ala Leu Val
mutated	
normal	TTT ATC AAG TAC CTC CCT GAA TGG ACT GCG
protein	Phe Ile Lys Tyr Leu Pro Glu Trp Thr Ala
mutated protein	Glu
normal	TGG CTC ATC TTG GCT GTG
protein	Trp Leu Ile Leu Ala Val
normal	ATT TCA GTA TAT G gtaaaacccaagactgataaattgtttgtc
protein	Ile Ser Val Tyr
normal	acaggaatgcc

**Figure 3:** The 165bp section of *PSEN1* that was amplified. The lines labeled “normal” indicate the sequence of the normal DNA. The lines labeled “protein” indicate the normal amino acid sequence. In red, on the line labeled “mutated”, the spot where adenine replaces cytosine is marked. In the line labeled “mutated protein”, the amino acid incorporated when the mutation is present is highlighted in red. The area that the restriction enzyme *DdeI* identifies is underlined, and the exact spot that it cuts is labeled with an arrow. In the mutated version, the DNA is cut at the arrow, creating two fragments that are 89 and 76 bp long.

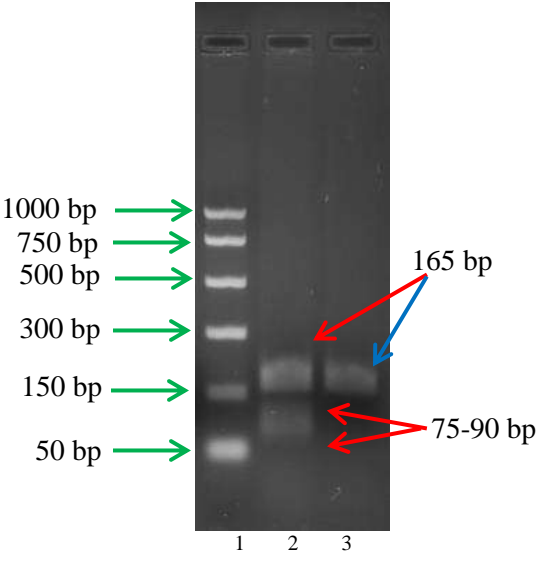
PCR of *PSEN2* yielded a 234 bp segment. This was digested with the enzyme *DPNII* from New England BioLabs in a reaction of 7 µL dI water, 10 µL PCR product, 2 µL 10x buffer from New England BioLabs, and 1 µL *DPNII* in a 37°C water bath left overnight. Once digestion was complete, tracking dye was added to the samples, which were loaded into a 3.5% agarose/TAE/ethidium bromide gel. This gel was made according to the procedure above, but instead combines 1.4g of agarose, 40mL of Tris-EDTA buffer, and 3µL of ethidium bromide. The samples were electrophoresed at 120 volts to allow for the DNA pieces to separate according to size, and then visualized under an ultraviolet light. It was expected that the normal DNA would be cut to 152 and 82 bp. Because the mutated DNA was heterozygous (the mutation is on one chromosome but not the other), it was expected that some of the DNA would be cut to 152 and 82 bp and some of the DNA would be cut to 152, 67, and 15 bp. The amplified area of *PSEN2* can be seen in figure 4.

normal	tcagC ATC TAC ACG CCA TTC ACT GAG GAC
protein	Ile Tyr Thr Pro Phe Thr Glu Asp
normal	ACA CCC TCG GTG GGC CAG CGC CTC CTC AAC
protein	Thr Pro Ser Val Gly Gln Arg Leu Leu Asn
mutated	
normal	TCC GTG CTG AAC ACC CTC ATC ATG ATC AGC
protein	Ser Val Leu Asn Thr Leu Ile Met Ile Ser
mutated protein	Ile
normal	GTC ATC GTG GTT ATG ACC ATC TTC TTG GTG
protein	Val Ile Val Val Met Thr Ile Phe Leu Val
normal	GTG CTC TAC AAG TAC CGC TGC TAC AAG
protein	Val Leu Tyr Lys Tyr Arg Cys Tyr Lys
normal	gtgagcccttgccctgcccctcagccaagcttctccgtctgcccacacc
normal	atggcggcaggcccctgaaacagccgctttaga

**Figure 4:** The 234bp section of *PSEN2* that was amplified. The lines labeled “normal” indicate the sequence of the normal DNA. The lines labeled “protein” indicate the normal amino acid sequence. In red, on the line labeled “mutated”, the spot where thymine replaces adenine is marked. In the line labeled “mutated protein”, the amino acid incorporated when the mutation is present is highlighted in red. The areas that the restriction enzyme *DPNII* identifies are underlined, and the exact spots that it cut are labeled with an arrow. In the mutated version, the DNA is cut at both arrows, creating three fragments that are 152, 67, and 15 bp long. In the normal version, the DNA is only cut at the second arrow, creating two fragments that are 152 and 82 bp long.

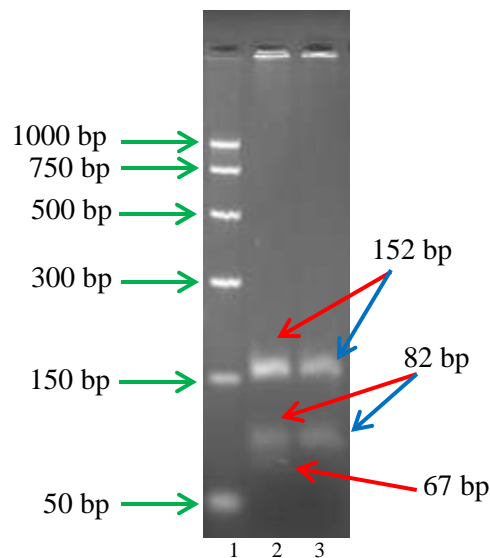
**Results**

The digestion products for *PSEN1* can be seen in Figure 5, where lane 1 is the PCR markers (1,000, 750, 500, 300, 150, and 50 bp), lane 2 is the mutated *PSEN1* DNA after digestion with *DdeI* showing products at approximately 165 and 75-90 bp, and lane 3 is the normal DNA after digestion with *DdeI* showing a single band at approximately 165 bp. As expected, the *DdeI* enzyme cut the mutated *PSEN1* DNA but not the normal DNA.



**Figure 5:** Photograph of agarose gel after successful *PSEN1* trial. Lane 1 shows the PCR markers with bands at 1000, 750, 500, 300, 150, and 50 bp (shown in green). Lane two shows the mutated DNA after digestion with *DdeI* with bands at 165 and 75-90 bp (shown in red). Lane 3 shows the normal DNA after digestion with *DdeI* with a band at 165 bp (Shown in blue).

The digestion products for *PSEN2* can be seen in Figure 6 where lane 1 is the PCR markers (as above), lane 2 is the mutated *PSEN2* DNA after digestion with *DpnII* showing products at approximately 152, 82, and 67 bp, and lane 3 is the normal DNA after digestion with *DpnII* showing products at approximately 152 and 82 bp. As expected, the *DPNII* enzyme cut the mutated *PSEN2* DNA twice but the normal DNA only once.



**Figure 6:** Photograph of agarose gel after successful *PSEN2* trial. Lane 1 shows the PCR markers with bands at 1000, 750, 500, 300, 150, and 50 bp (shown in green). Lane two shows the mutated DNA after digestion with *DPNII* with bands at 152, 82, and 67 bp (shown in red). Lane 3 shows the normal DNA after digestion with *DPNII* with a band at 152 and 82 bp (shown in blue).

## Discussion

As can be seen in figures 2 and 3, the mutant DNA was successfully distinguished from the normal DNA using PCR, restriction isotyping, and gel electrophoresis. Further research would be to use the same procedure to test samples whose sequence is not known to see if this technique can be implemented to accurately assess whether someone carries a mutation. The results of this further examination can only be confirmed by DNA sequencing, an expensive and time-consuming technology not currently available at this facility.

## Conclusion

This investigation was successful in that in both the *PSEN1* and *PSEN2* genes, the normal DNA could be distinguished from the mutated DNA through the use on enzymes. Because of this, this procedure could be used in genetic screening as a tool to determine if a patient is a carrier for one of these mutations linked to Alzheimer's disease. Since the key in preventing and slowing AD is minimizing risk factors, a patient's knowledge of a mutation as early as possible is crucial to their future, especially in families with a history of AD.

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## Biography

My name is Ashlee Junier and I am currently a senior preparing to graduate in May 2015 with Bachelor's degrees in Forensic Science, Biology, and Biotechnology. After graduation, I hope to pursue a Ph.D. in Genetics or Molecular Biology. I hope to eventually enter the biomedical research field, ideally continuing to research the genetics, causes, and mechanisms of Alzheimer's disease.



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