Detection of Mutations in the *PSEN1* and *PSEN2* Genes

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Introduction

Alzheimer's disease (AD) is the most common form of dementia worldwide, affecting millions of people and their families every year (1). Mutations in any one of a handful of genes leads to an increased risk for AD. Minimizing risk factors is crucial in slowing the progression of AD or even preventing it altogether, making identifying these genetic risk factors critical (2). Two common mutations linked to AD are located in the *presenilin 1* (*PSEN1*) and *presenilin 2* (*PSEN2*) genes (1). Both of these genes were examined in this study.

In the *PSEN1* gene, normal DNA was compared to DNA carrying a C>A substitution at nucleotide 56,338. As shown below, the enzyme *DdeI* cuts the DNA only at the sequence C/TGAG. If the C>A substitution is not present, *DdeI* will not cut the DNA.

Normal		C>A Substitution (shown in **red**)
---CTGCG---		---CTG**A**G---
---GACGC---		---GAC**T**C---
↓	*DdeI*	↓
---CTGCG---		---C TG**A**G---
---GACGC---		---GAC**T** C---

In the *PSEN2* gene, normal DNA was compared to DNA carrying an A>T substitution at nucleotide 15,033. As shown below, the *DPNII* enzyme cuts the DNA only at the sequence GATC. If the A>T substitution is not present, *DPNII* will cut PSEN2 once; with the mutation, *DPNII* cuts *PSEN2* twice.

Normal		A>T Substitution (shown in **red**)
---GAAC---		---GA**T**C---
---CTTG---		---CT**A**G---
↓	*DPNII*	↓
---GAAC---		--- GA**T**C---
---CTTG---		---CT**A**G ---

Materials and Methods

Both the *PSEN1* and *PSEN2* genes were amplified using PCR in 0.2µL Ready-to-Go PCR tubes from GE Healthcare. The PCR reaction was a total of 25µL containing 23µL of dI water, 1µL of primers (5 nm final concentration of each primer), and 1µL of DNA (2 nm final concentration) from Coriell Cell Repository. For *PSEN1*, the primer sequences were AAAGGTCCACTTCGACTCCA (L) and GGCATTCCTGTGACAAACAA (R). For PSEN2, the primer sequences were TCAGCATCTACACGCCATTC (L) and TCTAAAGGCGGCTGTTTCAC (R). The reaction containing tubes were placed in the thermocycler for PCR using the following program: 5 minutes at 95ºC for denaturation, thirty cycles of 95ºC, 56ºC, and 72ºC for 30 seconds each, and 10 minutes at 72ºC 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 µL dI water, 10 µL PCR product, 2 µL 10x buffer from New England Biolabs, and 1 µL *DdeI* in a 37ºC water bath left overnight. Once digestion was complete, the samples were loaded with tracking dye and electrophoresed on a 2% TAE/agarose/ethidium bromide gel at 120 volts. 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.

PCR of *PSEN2* yielded a 234 bp segment. This was digested with the enzyme *DdeI* 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 37ºC water bath left overnight. Once digestion was complete, the samples were loaded with tracking dye and electrophoresed on a 3.5% TAE/agarose/ethidium bromide gel at 120 volts. 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, 67, and 15 bp.

Results

The digestion products for *PSEN1* can be seen in Figure 1 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.

The digestion products for *PSEN2* can be seen in Figure 2 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, 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.

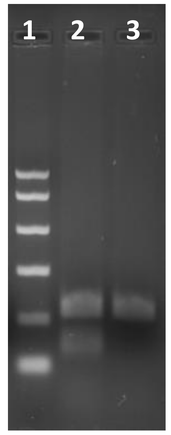

Figure 1

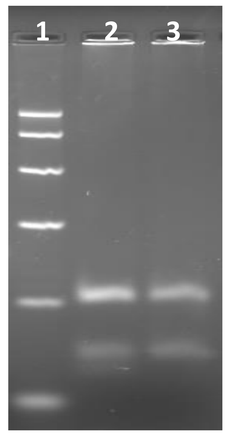

Figure 2

Conclusions

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.

References

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