Detection of Mutations in the PAH, NF1, and BRCA1 Genes

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Abstract

Several mutations occurring within the human genome can be easily detected through the use of a process known as PCR, or polymerase chain reaction. PCR allows scientists to take small fragments of DNA and amplify, or copy, them so that an adequate amount of the sample is available for further examination. This process generally only takes a few hours, and can provide scientists with millions of copies of the sample in question.

The specificity of the region the scientists can attempt to amplify depends on the primers used. Primers are short sequences of deoxyribonucleotides that are complementary to the beginning and end portions of the of the segment to be amplified through PCR. By developing primers specific to portions of certain genes, the presence of several disease-causing mutations can be examined in a sample of patients' DNA. In this report, primers were designed to identify specific mutations in the PAH, NF1, and BRCA1 genes. These primers allowed for distinctions to be made between DNA containing the mutations and DNA that was mutation-free.

Introduction

Phenylketonuria (PKU) can be caused by

this gene leave a person more susceptible to developing breast and/or ovarian cancers. Connections have been made between mutations within this gene and the development of these cancers within families, leading scientists to believe that it contributes to "inherited" cases of cancer (Ransburgh et al., 2010). Just as it is important to try to determine if someone's DNA contains disease-causing mutations in their PAH and NF1 genes, the same can be said for the BRCA1 gene. The mutation of interest within this gene causes the deletion of a 40 base pair segment, beginning at the gene's 1294th base pair.

Materials and Methods

DNA Extraction (for NF-1 Trials only)

Flat toothpicks were rubbed vigorously against the inside of the cheek in order to dislodge cells. The toothpicks were then immersed in individual 1.5 microcentrifuge

placed in a boiling water bath for 15 minutes. After 15 minutes, the tubes were removed from the boiling water, cooled and used for the PCR procedure.

PCR Procedure:

For each trial, 0.2 mL Ready-to-Go PCR tubes from GE Healthcare were prepared with 22 L of dH_2O, 2 L of primer mix, and 1 L of DNA.

The tubes for the PAH and BRCA1 trials were both thermocycled according to the following settings: 95°C for 10 minutes; 35 cycles of: 95°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds; 72°C for 10 minutes; hold at 4°C.

The tubes used for the NF1 trails were thermocycled according to the following settings: 95°C for 10 minutes; 35 cycles of: 95°C for 45 seconds, 57°C for 45 seconds, 72°C for 1.5 minutes; 72°C for 10 minutes; hold at 4°C.

T1/P

Digestion (for PAH trials only):ordi(7(l7 8997(:20 Td (°)Tj -0.004 Tc 0.004 Tw5uTc 0Tw5uTc 0Tw5u /P <m60Ct9.271.157 Td [(T

pairs that were supposedly present in the mutated genome. After careful research, it was determined that the information cited by both Coriell and the article itself were outdated, since the article was published over a decade before the human genome was even fully sequenced. It was then discovered that this article was referenced in a much more recent article, published in Clinical Chemistry in 2010. In this article, it was stated that the Alu insertion was located 44 base pairs upstream of the 42nd exon, not the 6th (Chouet al., 2010). With this newfound information, the predicted location of the mutation was then found using Genbank, which then provided a sequence that could be entered into PrimerBlast.

The primer sequences generated by PrimerBlast (listed in Table 2) were predicted to amplify a segment of DNA that was 275 base pairs long without the mutation. With the mutation, the DNA segment that would be amplified would be approximately 575 base pairs long. These results were clearly seen upon electrophoresis of the four amplified samples of DNA. The “normal” DNA only produced a prominent band in the 275 base pair range, while the DNA containing the insertion showed two bands; one in the 275 base pair range, and the other in the 575 base pair range. The two DNA samples extracted from Angelica and Jessica showed only one band each in the 275 base pair range (Figure 2). These acted as controls for the experiment, since it was known that neitherindividual suffered from Neurofibromatosis Type 1.

1 3 5 7 9

Figure 2. Photograph taken of the electrophoresed gel from the final successful NF-1 trial. Lane 1: Contained mutant NF-1 DNA, 2 bands seen, one was around 275 base pairs long, and the other was around 575 base pairs long; Lane 3: Contained normal DNA, one band around 275 base pairs long was seen; Lane 5: [illegible]

3 5 7

Figure 3. Photograph taken of the electrophoresed gel from the final successful BRCA1 trial. Lane 3: Contained mutant BRCA1 DNA, one band that was 205 base pairs long, and one band that was 245 base pairs long were seen; Lane 5: PCR marker; Lane 7: Contained normal DNA, only one bad that was 245 base pairs in length was seen.

Gene	Primer Sequences
PAH	Forward: 5' GTGATTTCCCGAAAGTGAGAGC 3'
	Reverse: 5' ACTTTCTGCAGGGCCATTGA 3'
NF-1	Forward: 5' ATCACTTTTCCTTTTGCCCTGT 3'
	Reverse: 5' CAGCATCAGCATGTAGCGTG 3'
BRCA1	Forward: 5'AGAAACTGCCATGCTCAGAGAATC3'
	Reverse: 5'ATGAGGATCACTGGCCAGTAAGTC3'

Table 2. Primers that successfully identified mutations in the PAH, NF-1 and BRCA1 genes.

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Biography

Jessica Imperato is currently a junior, and will graduate in May 2015 with dual B.S. degrees in Forensic Science and Biology. Originally from Thiells, New York, she hopes to pursue PhD in cellular and molecular biology, and eventually enter the clinical research field.