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Detection of LDL receptor mutations by PCR, PCR-RFLP and Southern analysis of DNA from Oragene®/saliva samples†

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The Oragene® self-collection kit is a non-invasive method of collecting DNA from saliva and it gives the same results as DNA from blood for the analysis of mutations in the LDL receptor gene by PCR, PCR-RFLP and Southern analysis.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder that occurs about one in 500 individuals in Western populations. It is primarily caused by mutations in the LDL receptor (LDLR) gene and over 770 mutations have been described worldwide[1]. Among French Canadians, the prevalence of FH heterozygotes has been estimated to be about one in 270[2]. Due to a genetic founder effect, five mutations in the LDLR gene account for approximately 83% of FH diagnosed in French Canadians[3]. These mutations are gene deletions of >15 kb and 5 kb, and three missense mutations: Trp_{66} → Gly (exon 3), Glu_{207} → Lys (exon 4), and Cys_{646} → Tyr (exon 14). Methods such as Southern blotting[4], PCR-RFLP[5], and PCR[6] have been used to detect these mutations in DNA from blood. Unlike blood, the Oragene self-collection kit is a non-invasive method for collecting DNA from saliva. The purpose of this study was to compare the detection of these five LDLR gene mutations in DNA from blood and DNA from Oragene/saliva samples.

Materials and methods

DNA collection and purification

Blood and saliva samples were collected from nine healthy French-Canadian volunteers and five patients who had been previously diagnosed with FH due to various mutations in the LDLR gene. Saliva was collected using the Oragene self-collection kit (DNA Genotek) and DNA was purified according to the prepIT™•L2P protocol[7]. Additional DNA was purified from 10 mL of whole blood using the QIAamp® DNA blood maxi kit (Qiagen). All of the samples were tested for five mutations: >15 kb and 5 kb deletions, and mutations in exons 3, 4, and 14.

>15 kb and 5 kb deletions

The >15 kb and 5 kb deletions in the LDLR gene were detected using two methods. First, 10 µg of genomic DNA from blood and Oragene were digested with the restriction enzymes XbaI and EcoRV, and analyzed with Southern blotting using the method of Ma et al. (1989). Second, the PCR assays described by Simard et al. (2004) were also used to test for both deletions.

Exon 3 and exon 4 mutations

Exons 3 and 4 were amplified by PCR, digested with BslI and MnlI respectively, and electrophoresed on 2.5% MetaPhor agarose gels (Cambrex) according to the method of Minnich et al. (1995).

Exon 14 mutation

Following the method of Vohl et al. (1995), mismatch primers were used to create a NlaIII restriction site in the exon 14 mutant but not in the normal allele. After digestion, the mutant allele gave two fragments of 163 and 33 bp in size, while the normal allele resulted in a 196 bp fragment. The fragments were separated by electrophoresis on 2.5% MetaPhor agarose gels.

Results

DNA yield

The average DNA yield was 159 µg from 2 mL of saliva collected with Oragene kits. The average $A_{260}/_{280}$ ratio was 1.94.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

Mutation detection

Table 1 summarizes the results of testing for the five mutations by PCR, PCR-RFLP, and Southern analysis. Figure 1 shows a sample result for PCR-RFLP analysis of the mutation in exon 3.

Patient #	Oragene results	Blood results
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	>15 kb	>15 kb
12	>15 kb	>15 kb
13	exon 14	exon 14
14	exon 3	exon 3

***Table 1**: Comparison of results from blood and Oragene for detection of >15 kb and 5 kb deletions, and mutations in exons 3, 4, and 14 of the LDLR gene.*

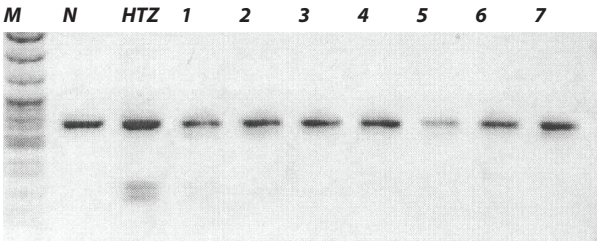

***Figure 1**: PCR-RFLP results for detection of the LDLR gene mutation in exon 3. Lanes 1 and 2 show results with DNA from blood for Patients #1 and 2. Lanes 3 to 7 show results with DNA from Oragene for Patients #1 to 5. (M=Marker, N=Normal, HTZ=Heterozygote).*

Discussion and conclusions

Patients #1 to 9 were healthy volunteers with no signs or symptoms of FH. As expected, they all tested negative for the five mutations using DNA from blood and Oragene. Patient #10 had the clinical characteristics of FH, but had previously tested negative for the five mutations with DNA from blood. The same negative result was seen again with DNA from Oragene/saliva samples. Patients #11 to 14 had been diagnosed with LDLR mutations with DNA from blood and the same results were seen with DNA from Oragene/saliva samples. Overall, sample collection was easier and non-invasive with Oragene, and the PCR, PCR-RFLP, and Southern blot results were the same as with DNA from blood.

References

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7 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.