A Patient With Desmoid Tumors and Familial FAP Having Frame Shift Mutation of the *APC* Gene

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Received: 13 Feb. 2016; Accepted: 22 Aug. 2016

Abstract- Desmoids tumors, characterized by monoclonal proliferation of myofibroblasts, could occur in 5-10% of patients with familial adenomatous polyposis (FAP) as an extra-colonic manifestation of the disease. FAP can develop when there is a germ-line mutation in the adenomatous polyposis coli gene. Although mild or attenuated FAP may follow mutations in 5' extreme of the gene, it is more likely that 3' extreme mutations haveamore severe manifestation of thedisease. A 28-year-old woman was admitted to the Cancer Institute of Iran with an abdominal painful mass. She had strong family history of FAP and underwent prophylactic total colectomy. Pre-operative CT scans revealed a large mass. Microscopic observation showed diffuse fibroblast cell infiltration of the adjacent tissue structures. Peripheral blood DNA extraction followed by adenomatous polyposis coli gene exon by exon sequencing was performed to investigate the mutation in adenomatous polyposis coli gene. Analysis of DNA sequencing demonstrated a mutation of 4 bpdeletions at codon 1309-1310 of the exon 16 of adenomatous polyposis coli gene sequence which was repeated in 3 members of the family. Some of them had desmoid tumor without classical FAP history. Even when there is no familial history of adenomatous polyposis, the adenomatous polyposis coli gene mutation should be investigated in cases of familial desmoids tumors for a suitable prevention. The 3' extreme of the adenomatous polyposis coli gene is still the best likely location in such families.

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Keywords: Desmoids; Familial adenomatous polyposis; APC gene; Mutation

Introduction

Desmoids tumors, also known as aggressive or deep fibromatosis, are rare tumors characterized by a monoclonal proliferation of myofibroblasts in muscles, tendons, and ligaments. In 2013, WHO classified them in the group of locally aggressive, non-metastasizing mesenchymal tumors (1). Desmoid tumors occur in 5-10% of the patients who have familial adenomatous polyposis (FAP) which is autosomal dominantly inherited (2). They develop as an extracolonic manifestation of the disease (3). They often reappear after local resection and can cause death through local infiltration of vital structures (4). FAP is typically characterized by thousands of adenomatous polyps of the colon throughout the 2nd and 3rd decade of life. Virtually, in all FAP patients, one or more of these adenomatous polyps progress to adenocarcinoma if prophylactic colectomy is not done in the second decade of life (5). The number of important genotypephenotype correlations have been determined for FAP families. Extra-colonic manifestations of FAP include duodenal adenomas, periampullary carcinomas, gastric gland polyps, desmoids tumors, osteomata, epidermoid cysts, and congenital hypertrophy of the retinal pigment epithelium (CHRPE) (6). FAP is caused by germline mutations in the adenomatous polyposis coli (APC) gene. Patients with mutations at the 5' extreme end of the gene (usually exons 3 and 4) have attenuated FAP, characterized by fewer than 100 adenomatous polyps, later onset of polyposis and later onset of colorectal cancer (7). Patients with CHRPE have mutations between exons 9 and codon 1444. Profuse polyposis (i.e. greater than 5,000 adenomatous polyps) has been shown

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to correlate with mutations between codons 1250 and 1464. Approximately, 10% of FAP families have one or more members who develop a desmoids tumor (2). In this paper, important aspects of this syndrome are reviewed, describing an example based on a well-documented clinical case.

Materials and Methods

A 28-year-old woman was admitted to our center, i.e. Cancer Institute of Iran, with a painful mass in the abdomen. During a physical examination, the mass was observed to be firm, lacking tenderness and fixed to the abdominal wall. The patient stated that the mass was gradually increasing in size. The patient hadarelevant family history of FAP but no history of smoking, drinking alcohol or taking any medication. The patient had ahistory of prophylactic total colectomy. Preoperative CT scans revealed a large mass (9.3 x 6.1 cm) with unclear borders of attenuation equal to that of muscle (Figure 1).



Figure 1. Abdominal CT scan shows mesenteric tumor (arrow)

No specific imaging features can distinguish desmoids tumors from other solid masses. However, the diagnosis of these tumors should be considered in patients with an abdominal mass, a history of previous abdominal surgery or injury and where there may be an association with FAP. So a definitive diagnosis was done with a histopathological analysis (8). Characteristically, there was diffuse fibroblast cell infiltration of the adjacent tissue structures (Figure 2).



Figure 2. Microscopic observation of mesenteric mass biopsies showingafibroblastic pattern

In addition, the immunohistochemical for muscle cell markers such as actin had been used to differentiatedesmoid from fibrosarcoma in this case. The familial survey showed several cases of desmoids tumors and gastrointestinal diseases, in the family. All the three members of this family with recurrent desmoids tumors had FAP that was detected at their early 20s. This woman had an uncle who had been treated for a colon cancer four years before her admission to our center. Also, he had three sons which the second one suffered from FAP as well. A few years after probands FAP resection, mesenteric desmoids recurrences were also treated surgically for them. For her mother and brother, desmoids tumors occurred at the ages of 40 and 16-year-old, respectively. Although her father and sister had a normal colonoscopy at ages 55 and 35-year-old, two maternal aunts and three other relatives (their children) were also reported to have FAP (Figure 3).



Figure 3. Four generation pedigree There are family members which may be asymptomatic

This study was approved by the ethics committee of Tehran University of Medical Sciences. A blood sample was taken from the patient and her family members after they signed informed consent. DNA was extracted from peripheral blood cells using standard procedures. Polymerase chain reaction amplification was used to amplify all 16 exons of the APC gene. This was done in a 25 μ l reaction volume using 100 ng of genomic DNA as template, 10 pmol of each primer, 2.5 μ l of 10X PCR buffer (Roche, Germany), 1.5 mM MgCl₂, 0.2 mMdNTPs and 0.2 U Taq polymerase (Roche, Germany). Samples were heated at 95° C for 5 min followed by 32 cycles (45 s at 94° C, 45 s at 55° C, and 50 s at 72° C) and a final extension period of 5 min at 72° C. PCR products were separated

On a 1.5% agarose gel and visualized by DNA stain. Primers for APC gene were designed by primer 3 software (0.4.0 version) http://bioinfo.ut.ee/primer3-0.4.0/primer3/. The polymerase chain reaction used right and left primers for the coding sequence of APC gene (Table 1).

| ExonForwardReverse15'-GCAGGGAAATTGAAACACTGAGA-3'5'-AAGACAGTGCGAGGGAAAAC-3'25'-TTTCATCCTCTTAGATGCTGCT-3'5'-AAATGCTAACTTTGCAAGAAGA-3'35'-AAGGTGCGTGGCTTTGAGAGT-3'5'-ACCAACACCCCAAATCGAGAG-3'45'-TTACCCTGACCAAGTGGGAC-3'5'-TGCAAAATGCTCTAAGTGTTAGC-3'55'-GAGAAGTTTGCAATAACAACTGATG-3'5'-TTGGCAACAGCCCATGATGACC3'65'-CATGCACCATGACTGACGAA-3'5'-TTGGAACGCGCAGAGCGAAACA-3'75'-AGGGCAACAGAGCGAGACT-3'5'-TGGTACTGAATGCTTCTGGAAAA-3'85'-CGTGCAGCTCTAATGCTCAA-3'5'-TGGATTTAAATTCTCTGAAAGCTGCGA-3'95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TTGGATGGAGCCACTGC-3'105'-CTGGAAAGGGAAGACGAGTGGT-3'5'-TTGAGTAGCACAAATGGCTGA-3'115'-GTGCAAGGGCAGATGGCATAA-3'5'-GGACGAGGAGAGCGA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GAACTGGAGGAATCCTTG-3'135'-TGACAAAGGAAACAGTGGCATAA-3'5'-GAACTGGAGGAATTCCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCCATCCA-3'155'-AGTGGAGGACGGGCAATAG-3'5'-TCTTCAGAACATGCACTCCA-3'16-15'-TGTTATCGCATACACATTGTGCCTT-3'5'-TCTTCAGAATAGGATTCATCGAGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATCAAACGAG-3'16-35'-GAGCGAAACTGCCCAACAATTATAGCA-3'5'-TGTTTGGCCAGGGGGCACTCCTG-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-TGTTGGCCAGGGGAAAAA-3'16-55'-GAGCGAAACTCCCCCCAA-3'5'-TGGTGGCCAGAAATAA-3'16-65'-CCAAAGGGAAAGAGGCCAACAAATAAAA-3'5'-TGGTGCTGGGGAAAAA-3'16-75'-CCCAAAGGGAAAGAGG | Table 1. Primers for different exons of APC gene | | |
|--|--|---------------------------------|----------------------------------|
| 25'-ITTCATCCTCTTAGATGCTGCT-3' 35'-AAAGGTGCGTGCTTTGAGAGC-3' 5'-ACCAACACCCAAATCGAGAGA-3' 5'-ACCAACACCCAAATCGAGAGA-3' 5'-ACCAACACCCAAATCGAGAGA-3' 5'-ACCAACACCCAAATCGAGAGA-3' 5'-CCAGGCAGACAGAGCATGACGAACAGACGAGACA-3' 5'-CCAGGCAACAGAGCGAGACT-3' 5'-CCAGGCAGCATGACGTCAATGCTCAA-3' 5'-CGGTATTTAAATTCTCTGAAAGAACA-3' 5'-TGGTACTGAATGCTTCAGGAGAACA-3' 5'-TGGTACCAACAGGCAGACTGACGTT-3' 5'-TGGTACCAACAGGCGAGACT-3' 5'-TGGTACCAACAGGCGAGACA-3' 5'-TGGTACCAACGGCGAGAACAGATGGCTA-3' 5'-TGGTACCAACGGCGGAGAACAGATGGCAA-3' 5'-TGGTACGAACAGAGAGACA-3' 5'-TGGTACGACACAGAGGAGAGACA-3' 5'-TGGTACGACACAAATGGCTGA-3' 105'-CGAGAGGGTGAGCCA-3' 5'-TGGTACGACGCCATGGAA-3' 5'-TGGTACCAACAGAGGAGAGCA-3' 5'-TTTTGCACAACAGAGGAGAGACA-3' 5'-GATCAGACCACTGCA-3' 5'-GATCAGACCACTGCAACTGCCA-3' 5'-TTTTGGCTAAAACTTTCAGTCATGA-3' 16-1 5'-GGTGAGGAGAACCAACAATGGCGCA-3' 5'-TGTTCAGGACAACGAAACGAATAGCA-3' 5'-TGTTCAGGAATAGGACTACCAACAATTATAGCA-3' 5'-TGTTGCGGGAGGAAACTGGCAGA-3' 5'-TGTTGGCGGGGCTCCCGA-3' 16-4 5'-GAAGAGAGACCAACAAATCACCATGGCAGA-3' 5'-TGTTGCAGACCAGGGAAACTGCCCAACAA 16-5 5'-CCAAAGGGAAACTGCCCAACAAATTATAGCA-3' 5'-TGTTGGCAGGGGCACTGCAACAACAAATCAACCAATCAGC-3' 5'-TGTTGGCAGGGGCACTGCAACAACAATCAACCAATCAGC-3' 5'-TGTTGGCAGGAACTGGCGGCACAACAACAACAATCAGC-3' 5'-TGTTGGCAGGCAGCAACTGGCAGAAAGCA-3' 16-6 5'-CCCAAAGGGAAAAGGGCAGAAAAA-3' 5'-TGTTGGCAGGCAGCAACTGGCGAAAAC3' 5'-TGTTGGCAGGCAGCACTGGGAGAAACA-3' 5'-TGTTGGCAGGGGCACAACAACAACAATCAGC-3' 5'-TGTTGGCAGGCAGCTTGATACCCCTGA-3' 5'-AGCCAACCAATGGCCAACCAACAACAACAACAACAACAACAACAACAACAA | Exon | Forward | Reverse |
| 35'-AAGGTGCGTGCTTTGAGAGT-3'5'-ACCAACACCCAAATCGAGAG-3'45'-TTACCCTGACCCAAGTGGAC-3'5'-ACCAACACCCAAATCGAGAG-3'55'-GAGAAGTTTGCAATAACAACTGATG-3'5'-TGCAGGCCTAAAGTTGGGTAAAA-3'65'-CATGCACCATGACTGACGA-3'5'-TCAGGCCTAAAGTTGGGTAAAA-3'75'-AGGGCAACAGAGCGAGACT-3'5'-CGGTATTTAAATTCTCTGAAAGAACA-3'85'-CCATTCTGCAGCTTTAATGCTCAA-3'5'-TGGTACTGAAGGCACACGCACTGC-3'95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TGAGTAGCACAAATGGCTGA-3'95'-CCGTGAAGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGGGGAGAACCA-3'105'-CTGGAAAAGGTAGATGGTG-3'5'-TTTTTGCTACACAGAGGAAGAACA-3'115'-GTCAAGGGCAGAAACTGGCATAA-3'5'-TTTTTGCTACACAAGAGGAAGAACAGATAGCA-3'125'-GGGTGGAGAAAACTGGCATAA-3'5'-GCACAGGTTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GCACAGGGAGATTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GAATCAGACCACTGCACTCCA-3'155'-AGTGAGGAAGGACGAATAGC3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTCATGCATACACATTGTGCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-TGTTGGCATGGCGGAAAAA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-TGTTGGCATGGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCCTCCAA-3'5'-TGTTGGCATGGGGAAAATAA-3'16-65'-CCCAAAGGGAAAAGGCCAACAAATCAGC-3'5'-TGTGGCATGGGGAAAAAA-3'16-75'-CCCAAAGGGAAAGAGCCAACAAATCAGC-3'5'-TTGAGCCACCTATTGGGAACA-3'16-85'-AGCCAACTGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATGA-3'16-95'-AGACAAGGCCAACCATCAGCA-3' <th>1</th> <th>5'-GCAGGAAATTGAAACACTGAGA-3'</th> <th>5'-AAGACAGTGCGAGGGAAAAC-3'</th> | 1 | 5'-GCAGGAAATTGAAACACTGAGA-3' | 5'-AAGACAGTGCGAGGGAAAAC-3' |
| 45'-TTACCCTGACCCAAGTGGAC-3' 5'-GAGAAGTTTGCAATAACAACTGATG-3' 65'-GAGAAGTTTGCAATAACAACTGATG-3' 5'-TCAGGCCTAAAGTTGGGTAAAA-3'65'-CATGCACCATGACTGACGTA-3' 5'-AGGGCAACAGAGCGAGACT-3' 85'-GTTGCTCAGCAGCCATGATA-3' 5'-GTGGAACGCTCTAATGCTCAA-3' 5'-TGGTACTGAATGCTTCTGGAAA-3'75'-AGGGCAACAGAGCGAGACT-3' 5'-CCGTTCTGCAAGTGCTCTGGAAACAACA-3' 95'-CCGTGCAACGCCTTAATGCTCAA-3' 5'-TGGTAGCACAAATGCTTCTGGAAA-3' 5'-TGGTAGCACAAATGGCTGA-3'95'-CCATTCTGCAAGTGAGTGGTT-3' 5'-TGGAAGGCAGAGGAGGTTTCCGGTTT-3' 105'-CTGGAAAGGTTTCCGGTTT-3' 5'-TGACAAAGGAAGAACAGATAACCA-3' 5'-TGTTACGCACAAATGGCTGA-3'105'-CTGGAAAGGAACAGATAGCGCATAA-3' 5'-GGGTGGAGAAACTGGCATAA-3' 125'-TGGAAAGGAAGAGAACAGATAACCA-3' 5'-GGACGAGGATTGCTTGA-3'115'-GTCAAGGGAAGAACAGATAGCA-3' 5'-GGACGAGGATTGCTGA-3'5'-GAACGGGGAGAGCAGAACAGATAGCA-3' 5'-GCACAAGGGAGGATTGCTTGA-3'125'-GGGTGAAGAGAACAGATAGCA-3' 5'-TGTTACTGCAACACTGCACTCCA-3' 145'-ATTACAGGCGTGAGTCACCA-3' 5'-TGTTACTGCAACATGGACTGCCTT-3' 5'-TCTTCAGAATAGGATTCAATCGAGA-3'16-15'-TGTTACTGCATACACATTGTGACC-3' 5'-TGTTGCCAACAATGGCTCTCCTG-3' 5'-TCTTCAGAATAGGAATCACACAATTATAGCA-3' 5'-TTTTGGCATAACAGTGGGGTCTCCTG-3'16-25'-ACTGCCAACAACAACAAATATATAGCA-3' 5'-TGTTGGCATGGCAGAAATAA-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3' 5'-TGTGGCATGGCAGCAACATCAGC-3'16-45'-GAAGAGAGACCAACAAATATATAGCA-3' 5'-TGCAAGCGAGCAGAATAA-3' 5'-TGGATGGCAGAAAAAA-3'16-55'-GAAGGAGACCAACAAATCAGC-3' 5'-TGGATTGTGGCAGAAAAA-3'16-65'-CCAAAGGAAAAGGCCAACAAACAA-3' 5'-TGGACCACCACTATTTGGGGAAAA-3'16-75'-ACCTCCAACCAAATCAGCC-3' 5'-AGCACCACCTATTGGGGACGA-3'< | 2 | 5'-TTTCATCCTCTTAGATGCTGCT-3' | 5'-AAATGCTAACTTTTGCAAGAAAGA-3' |
| 55'-GAGAAGTTTGCAATAACAACTGATG-3' 65'-TCAGGCCTAAAGTTGGGTAAAA-3' 5'-GTGCCAGCCATGACTGACGAA-3' 5'-GGTGCTCAGCAGCCATGATA-3' 5'-GGTGCTGAGCCACGGCCATGATA-3' 5'-CGGTATTTAAATTCTCTGAAAGAACA-3' 5'-TGGTACTGAATGCTTCGGAAA-3' 5'-TGGTACTGAAGCCACTGC-3' 5'-TGGTAGCAGCAAATGGCTGA-3' 105'-CTGGAAAGGTTTTCCGGTTT-3' 5'-TGACAAGGCAGAGTGAGTGGT-3' 5'-TGACAAAGGAAGAACAGATAGCA-3' 5'-TGACAAAGGAAGAACAGATAGCA-3' 5'-GGACAGGGAGGAGGAACTGGCAGAAACGGATAGCA-3' 5'-GGACAGGGAGGAGGAACTGGCATAA-3' 5'-GGACAGGGAGGAGGACGAGCAATGG-3' 5'-GGACAGGGAGGACTGCTTGA-3' 125'-GTCAAGGCGTGAGTACACA-3' 5'-GGACAGGGAGGACGGCAATGG-3' 5'-GGACGGGGAGGGACTGCTTGA-3' 5'-GGATCTAGGCACTGCACTCCA-3' 5'-GGATCTGGCAACATGCCTTGA-3' 5'-TTTTGGCTTAAAACTTTCATGCATTC-3' 5'-TCTTCAGAATAGGATTCAATCGAGC-3' 5'-TCTTCAGAATAGGATTCAATCGAG-3' 5'-TCTTCAGAATAGGATTCAATCGAG-3' 5'-TGTTGCTGGCAGAAACAACAATTATAGCA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCTGGAAACA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCTGGGAAAAA-3' 5'-TGTTGGGTGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGTTGGCATGGCAGAAAAA-3' 5'-TGACCCACCTATTGGGATGA-3' 16-10 5'-AGCCAAGCCACCTTGTGGAAAT-3' 5'-GAGGCGAAACAACTCGCC-3' 5'-GAGCGAGCTGCCATTGATACCCCAACCAACTTGGCAAA-3' 5'-TGACCCACCTATTGGGATCA-3' 5'-GAGCCAAGCCACCTACTGTAAGAA-3' 5'-GAGCGAGGCTGCCATTGATGAA-3' 5'-GAGCCAGCGTGCCATTGATAA-3' 16-11 5'-TCCCAAGCCCACCTACTGTGAAAA-3' 5'-CAGCAGGGGCCAACTTGA-3' 5'-GAATGGCGCATTGATCCCCAACCAACTTAGAA-3' 5'-CAGCAGGGGCCCATTGATGAA-3' 5'-CAGCAGGTGCCATTGATAA-3' 5'-GAATGGCGCACTTAGAAA-3' 5'-GAATGGCGCACTTGATGA-3' 5'-GAATGGCGCACTTGATGA-3' 5'-GAATGGCGCCACTTGATGA-3' 5'-GAATGGCGCACTTGATGA-3' 5'-GAACGGGGCCAACTTAA-3' | 3 | 5'-AAGGTGCGTGCTTTGAGAGT-3' | 5'-ACCAACACCCAAATCGAGAG-3' |
| 65'-CATGCACCATGACTGACGTA-3'5'-GTTGCTCAGCAGCCATGATA-3'75'-AGGGCAACAGAGCGAGACT-3'5'-CGGTATTTAAATTCTCTGAAAGAACA-3'85'-CGTGCAGCTCTAATGCTCAA-3'5'-TGGTACTGAATGCTTCTGGAAA-3'95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TTACAGGTGTGAGCCACTGC-3'105'-CTGGAAAGGTTTCCGGTTT-3'5'-TTACAGGTGTGAGCCACTGC-3'115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGAGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTGGGAGGATGCTTGA-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GAACTGGCACACTGCACTG-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GTTGGATGAGCACCACTGCACTGA-3'155'-AGTGAGGGACGGCAATAGC3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTCATGCCATACACATTGTGACC-3'5'-TCTTCAGAATAGGATTCCATGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCATGCCATAACACATTGTGACC-3'5'-TGTTGGCATAGAGGGGTCTCCTG-3'16-45'-GAGCGAAATTCCCCTCCAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-65'-CCCAAAGAGGAAAAGTCACAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGTGGCAGGAACTGGGGACTAA-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCATGAGCAGAA3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-AGCAGCAGCAGCTGAGGACTA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-CAGCAGGTGCCATTTGATAA-3' <th>4</th> <th>5'-TTACCCTGACCCAAGTGGAC-3'</th> <th>5'-TGCAAAATGCTCTAAGTGTTAGC-3'</th> | 4 | 5'-TTACCCTGACCCAAGTGGAC-3' | 5'-TGCAAAATGCTCTAAGTGTTAGC-3' |
| 75'-AGGGCAACAGAGCGAGACT-3'5'-CGGTATTTAAATTCTCTGAAAGAACA-3'85'-CGTGCAGCTCTAATGCTCAA-3'5'-TGGTACTGAATGCTTCTGGAAA-3'95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TGAGTAGCACAAATGGCTGA-3'105'-CTGGAAAGGTTTTCCGGTTT-3'5'-TGAGTAGCACAAATGGCTGA-3'115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGAGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GGAAGTGGGAGGAGTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GAACTGGCAACATGCTCCA-3'155'-AGTGAGGGACGGCGAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTATGCATACACATTGTGACC-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTGCTGGA-3'16-45'-GAAGAGAGACCAACAAATAAGCA-3'5'-TCTTGGAATAGGGGTCTCCTG-3'16-55'-GAGCGAAATCCCCTCCAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-65'-CCAAGAGAAGAGCAGAAAAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGTTGGCATGGCAGAAAAA-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-TGTGGCAGGAGCAGCAGAAG-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-GAGTTGTGGCCTGGGAACTA-3'16-105'-AGCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCCAAGCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-CAGCAGGTGCCATTTG-3' | 5 | 5'-GAGAAGTTTGCAATAACAACTGATG-3' | 5'-TCAGGCCTAAAGTTGGGTAAAA-3' |
| 85'-CGTGCAGCTCTAATGCTCAA-3'5'-TGGTACTGAATGCTTCTGGAAA-3'95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TTACAGGTGTGAGCCACTGC-3'105'-CTGGAAAGGTTTTCCGGTTT-3'5'-TGAGTAGCACAAATGGCTGA-3'115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGAGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GAACTGGGAGGAGGATTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GAACTGGCACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTGCCA-3'16-45'-GAAGAGAGACCAACAACAAATTATAGCA-3'5'-TGTTGGCATGGCGGTCTCCTG-3'16-55'-GAGCGAAAAGGGCAGAAAAA-3'5'-TGATTTTGGGTGCAGA-3'16-65'-CCCAAAGAGAAGAGGCAGAAAAA-3'5'-TTTGGATGACTGGGGACAA-3'16-75'-ACCTCCAACCAACAATCAGCC-3'5'-TGATTTTGGGTGCAGA-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-TGGCAGCAGCAGCAGCAGCTGATGAA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCATTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-CAGCAGCGCTTAGGACTTG-3' | 6 | 5'-CATGCACCATGACTGACGTA-3' | 5'-GTTGCTCAGCAGCCATGATA-3' |
| 95'-CCATTCTGCAGTTTAATGCTCA-3'5'-TTACAGGTGTGAGCCACTGC-3'105'-CTGGAAAGGTTTTCCGGTTT-3'5'-TGAGTAGCACAAATGGCTGA-3'115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGAGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GCACAGGTGGGAGGATTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-CTGGATTTACTGCTTGATCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCA-3'16-45'-GAAGAGAGACCAACAAAATTATAGCA-3'5'-TGTTGGCATGGCAGAAAATAA-3'16-55'-GAGCGAAAAGGGCAGAAAAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAAGAGAAAGAGGCAGAAAAA-3'5'-TTTTGGATGACTGGGGAAAAA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTTGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAAATCAGC-3'5'-TGACCCACCTATTTGGGATGA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATGA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGCTGGGACCTA-3'16-115'-TCCAAGCCAACCTTAAGAA-3'5'-GAGCTGGGGCCTTAGGACCTA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-CAGCAGGCGCTTAGGACCTTAG-3' | 7 | 5'-AGGGCAACAGAGCGAGACT-3' | 5'-CGGTATTTAAATTCTCTGAAAGAACA-3' |
| 105'-CTGGAAAGGTTTTCCGGTTT-3'5'-TGAGTAGCACAAATGGCTGA-3'115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GAAGTGGGAGGAGTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCA-3'16-45'-GAAGAGAGACCAACAAAATTATAGCA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-55'-GAAGCGAAAAGAGGCAGAAAAA-3'5'-TGATTTTGGTGGGGGACCAGA-3'16-65'-CCAAAGAGAAAAGAGGCAGAAAAA-3'5'-TGATTTTGGTGGGGGAAAAA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTGGATGACTGGGGAAAAA-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGCAGAAAA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATGA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAAGTGGCCATGATGAA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-GAAGTGGCCATTGGACAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 8 | 5'-CGTGCAGCTCTAATGCTCAA-3' | 5'-TGGTACTGAATGCTTCTGGAAA-3' |
| 115'-GTCAAGGGCAGATGAGTGGT-3'5'-TTTTTGCTACACAGAGGAAGCA-3'125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GAAGTGGGAGGACTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCCA-3'16-45'-GAAGAGAGACCAACAAAATTATAGCA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-55'-GAGCGAAAATCTCCCTCCAA-3'5'-TGATTGTGGCATGGCAGAAATAA-3'16-65'-CCAAAGAGAAAAGAGGCAGAAAAA-3'5'-TGATTTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTGGATGACTGGGGACTGAGAA-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGCAGCAGAAAG-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATGA-3'16-105'-AGCCAAGCCAACCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTGG-3' | 9 | 5'-CCATTCTGCAGTTTAATGCTCA-3' | 5'-TTACAGGTGTGAGCCACTGC-3' |
| 125'-GGGTGGAGAAACTGGCATAA-3'5'-GCACAGGTTTTATCAGTCATTG-3'135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GCACAGGTTTTATCAGTCATTG-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GAAGTGGGAGCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-GATCAGACCACTGCACTCCA-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-ACTGGCATGGCAGAAATAA-3'16-55'-GAGCGAAAATCCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAAGAGAAAAGAGGCAGAAAAA-3'5'-TGATTTTGTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGATTTTGGTGGGGACCTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGCAGAAAG-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-AGCAGCAGCAGCAGCTTGATGTA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTGG-3' | 10 | 5'-CTGGAAAGGTTTTCCGGTTT-3' | 5'-TGAGTAGCACAAATGGCTGA-3' |
| 135'-TGACAAAGGAAGAACAGATAGCA-3'5'-GAAGTGGGAGGATTGCTTGA-3'145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-ACTGGCATGGCAGAAATAA-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGATGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGCAGAAAAG-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATGA-3'16-105'-AGCCAAGCCAACCATCAGGAAA-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTGG-3' | 11 | 5'-GTCAAGGGCAGATGAGTGGT-3' | 5'-TTTTTGCTACACAGAGGAAGCA-3' |
| 145'-ATTACAGGCGTGAGTCACCA-3'5'-GATCAGACCACTGCACTCCA-3'155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTGGCGGGTCTCCA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-AACATGAGTGGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGATGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCATGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-GAGCTTGGCCAGCAGCAG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTGG-3' | 12 | 5'-GGGTGGAGAAACTGGCATAA-3' | 5'-GCACAGGTTTTTATCAGTCATTG-3' |
| 155'-AGTGAGGGACGGGCAATAG-3'5'-TTTTGGCTTAAAACTTTCATGATT-3'16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTGTCCA-3'16-45'-GAAGAGAGACCAACAACAAATTATAGCA-3'5'-ACATGAGTGGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCATGATGA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-GAGCTTGTGCCTGGGACCTA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCTTTCCTCAGGTGCTA-35'-GAATGGCCGCTTAGGACTTGG-3' | 13 | 5'-TGACAAAGGAAGAACAGATAGCA-3' | 5'-GAAGTGGGAGGATTGCTTGA-3' |
| 16-15'-TGTTACTGCATACACATTGTGACC-3'5'-CTGGATTTTCTGTTGCTGGA-3'16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTGGCA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-ACATGAGTGGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGATTTTGTTGGGGGCAGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGCAGAAAG-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCAACCTATTTGGGATGA-3'16-105'-AGCCAAGCCAACCTTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGAGCGCTTAGGACTA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCCGCTTAGGACTTG-3' | 14 | 5'-ATTACAGGCGTGAGTCACCA-3' | 5'-GATCAGACCACTGCACTCCA-3' |
| 16-25'-ACTGGCAACATGACTGTCCTT-3'5'-TCTTCAGAATAGGATTCAATCGAG-3'16-35'-TGTTCTATGCCTTATGCCAAT-3'5'-CGGTTTTACTGCTTTGTCCA-3'16-45'-GAAGAGAGACCAACAACAATTATAGCA-3'5'-ACATGAGTGGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGATTTTGTTGGGGGACAGAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCAGTGATA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCAACCATCTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 15 | 5'-AGTGAGGGACGGGCAATAG-3' | 5'-TTTTGGCTTAAAACTTTCATGATT-3' |
| 16-35'-TGTTCTATGCCTTATGCCAAAT-3'5'-CGGTTTTACTGCTTTGTCCA-3'16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-AACATGAGTGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGAAAAG-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAAACCAACAATCAGC-3'5'-AGCAGCAGCAGCATGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCAACCATCTTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTGG-3' | 16-1 | 5'-TGTTACTGCATACACATTGTGACC-3' | 5'-CTGGATTTTCTGTTGCTGGA-3' |
| 16-45'-GAAGAGAGACCAACAAATTATAGCA-3'5'-AACATGAGTGGGGTCTCCTG-3'16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTGGATGACTGGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCATGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCATTTTGGGATGA-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 16-2 | 5'-ACTGGCAACATGACTGTCCTT-3' | 5'-TCTTCAGAATAGGATTCAATCGAG-3' |
| 16-55'-GAGCGAAATCTCCCTCCAA-3'5'-TGTTGGCATGGCAGAAATAA-3'16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCATGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACTTTTGTGGCTGGGACTG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 16-3 | 5'-TGTTCTATGCCTTATGCCAAAT-3' | 5'-CGGTTTTACTGCTTTGTCCA-3' |
| 16-65'-CCAAGAGAAAGAGGCAGAAAAA-3'5'-TGATTTTTGTTGGGTGCAGA-3'16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCTTGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 16-4 | 5'-GAAGAGAGACCAACAAATTATAGCA-3' | 5'-AACATGAGTGGGGTCTCCTG-3' |
| 16-75'-CCCAAAGGGAAAAGTCACAA-3'5'-TTTGGATGACTGGGGAAAAG-3'16-85'-ACCTCCAACCAACAATCAGC-3'5'-AGCAGCAGCAGCAGCTTGATGTA-3'16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTG-3' | 16-5 | 5'-GAGCGAAATCTCCCTCCAA-3' | 5'-TGTTGGCATGGCAGAAATAA-3' |
| 16-8 5'-ACCTCCAACCAACAATCAGC-3' 5'-AGCAGCAGCAGCTTGATGTA-3' 16-9 5'-AGAACATGGTCTATCCCCTGA-3' 5'-TGACCCACCTATTTGGGATG-3' 16-10 5'-AGCCAAGCCATCTGTGAAAT-3' 5'-GAGTTTGTGCCTGGGACCTA-3' 16-11 5'-TCCAAGCCCAACCTTAAGAA-3' 5'-CAGCAGGTGCCATTTGATAA-3' 16-12 5'-GACCGTTTCCTCAGGTGCTA-3 5'-GAATGGCGCTTAGGACTTTG-3' | 16-6 | 5'-CCAAGAGAAAAGAGGCAGAAAAA-3' | 5'-TGATTTTTGTTGGGTGCAGA-3' |
| 16-95'-AGAACATGGTCTATCCCCTGA-3'5'-TGACCCACCTATTTGGGATG-3'16-105'-AGCCAAGCCATCTGTGAAAT-3'5'-GAGTTTGTGCCTGGGACCTA-3'16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTTG-3' | 16-7 | 5'-CCCAAAGGGAAAAGTCACAA-3' | 5'-TTTGGATGACTGGGGAAAAG-3' |
| 16-10 5'-AGCCAAGCCATCTGTGAAAT-3' 5'-GAGTTTGTGCCTGGGACCTA-3' 16-11 5'-TCCAAGCCCAACCTTAAGAA-3' 5'-CAGCAGGTGCCATTTGATAA-3' 16-12 5'-GACCGTTTCCTCAGGTGCTA-3 5'-GAATGGCGCTTAGGACTTTG-3' | 16-8 | 5'-ACCTCCAACCAACAATCAGC-3' | 5'-AGCAGCAGCAGCTTGATGTA-3' |
| 16-115'-TCCAAGCCCAACCTTAAGAA-3'5'-CAGCAGGTGCCATTTGATAA-3'16-125'-GACCGTTTCCTCAGGTGCTA-35'-GAATGGCGCTTAGGACTTTG-3' | 16-9 | 5'-AGAACATGGTCTATCCCCTGA-3' | 5'-TGACCCACCTATTTGGGATG-3' |
| 16-12 5'-GACCGTTTCCTCAGGTGCTA-3 5'-GAATGGCGCTTAGGACTTTG-3' | 16-10 | 5'-AGCCAAGCCATCTGTGAAAT-3' | 5'-GAGTTTGTGCCTGGGACCTA-3' |
| | 16-11 | 5'-TCCAAGCCCAACCTTAAGAA-3' | 5'-CAGCAGGTGCCATTTGATAA-3' |
| 16-135'-CAGCGCAGATAGCACTTCAG-3'5'-AAGCAGGCTGGGTAAACTTG-3' | 16-12 | 5'-GACCGTTTCCTCAGGTGCTA-3 | 5'-GAATGGCGCTTAGGACTTTG-3' |
| | 16-13 | 5'-CAGCGCAGATAGCACTTCAG-3' | 5'-AAGCAGGCTGGGTAAACTTG-3' |

Table 1. Primers for different exons of APC gene

Results

DNA derived from blood was used for polymerase chain reaction to amplify different exons of APC gene. Inthecase of exon 16, it was divided into 13 slices for polymerase chain reaction amplification. Since the designed primers had the same annealing temperature, all products were amplified using one program at the same time. The whole coding sequence and splicing junctions of the APC gene were systematically sequenced (Sanger 3130, ABI, USA). An independent DNA sample evaluated and controlled the mutations after a computer analysis using the Chromas software. Direct sequencing of genomic DNA revealed an identical frameshift mutation (4 bp deletion at codon 1309-1310) of the exon 16 of APC gene sequence (Figure 4). All 3 members of the family (proband, her mother, and her brother) were detected with frameshift mutation. However, investigation on the only firstdegree family member who was not affected (i.e. her sister) did not show the same mutation in sequencing analysis.



Figure 4. Chromatogram has shown 4 base pair deletion of codon 1309-1310 in exon 16 of APC gene which is marked on the sequence Vertical arrow corresponds to the mutation point, andthehorizontal arrow shows the frame shift.

We recently encountered an Iranian family with autosomal dominantly inherited desmoids tumors. Members of this family had lots of colon polyps, multifocal desmoids, and desmoids in abdominal locations. This prompted us to examine the proband for a mutation of the APC gene. We described a family with a protein truncating mutation in the 3' end of the APC gene. Desmoids tumors can cause death by compressing vital structures, and they are a major cause of morbidity and mortality in FAP patients (9).

The family examined in this study confirms previous studies' findings that certain mutations at the 3' end of the APC gene are associated with a high penetrance for the development of desmoids tumors (often atypically located) but mild or not polyposis.

This study introduces a family in which there is a solid penetrance of familial desmoid tumors either secluded or related with a common form of FAP. A pathogenic mutation has been found at the 3' end of the APC gene by molecular studies. Up to now, hundreds of different mutations in the APC gene have been identified. Most of these mutations have been located between codons 1250 and 1400, within exon 15. They lead to the synthesis of a truncated protein.

In particular, mutations in the exon 16 mutation hotspot normally cause profuse colonic polyposis. However, mutations located at the 5' end of the gene (i.e. before codon 168) or at the 3' end the gene (i.e. after codon 1580) are usually because of attenuated polyposis. The sites of these mutations determine the colorectal adenomatous density, in addition to the presence or absence of extra-colonic manifestations of the disease (10). Therefore, FAP and desmoids tumors association is frequently encountered in mutations located after codon 1444 (11,12). Patients with mutations situated on the 3' end of codon 1444 have 12 times more occurrence of desmoids tumors than those with mutations situated on the 50 end of codon 1444 (13). Eccles and colleagues have reported a high frequency of desmoids tumors withthevery late onset and incomplete penetrance of intestinal polyposis in a large kindred with an APC mutation at codon 1924 (14).

To date, more than 450 germ-line mutations of the APC gene have been described in FAP kindred's (more than 95% of the codons and truncation of the 312-kD wild-type protein into shorter polypeptides) (15). In general, premature chain-terminating mutations

approximately between codon 300 (exon 9) and codon 1600 lead to the more "classical" form of FAP with more than 1,000 colonic polyps and a variable incidence of extra-colonic manifestations with the only exception of CHRPE that are consistently present up to codon 1444 (12). A cluster of mutations delimited by residues 1250 and 1464 was found to be associated with a profuse phenotype with more than 5,000 colorectal adenomatous polyps per affected individual (16).

Mutations in the region between codons 1445 and 1578 result in severe desmoids, though never with 100% penetrance, but also osteomas, epidermoid cysts, and polyps of the upper GI tract, without CHRPE (11). Spirio and colleagues have shown that chainterminating mutations located at the 5' end of the APC gene lead to a milder FAP phenotype, i.e. attenuated familial adenomatous polyposis, characterized by fewer polyps (<100) and a delayed age at onset (17). These early APC mutations are predicted to result in extremely short and presumably unstable truncated polypeptides. Germ-line mutations located downstream of codon 1600 are extremely rare and seem to result in a lower colorectal tumor multiplicity and variable extra-colonic phenotypes (18). The mutation in codon 1309 is the most common mutation of APC gene that related to early onset of colorectal cancer (19).

The presented familial case plus those described in the literature, conclude that in cases of familial desmoids tumors an APC gene mutation must be looked for. This is true even in the absence of a personal or familial history of adenomatous polyposis for a suitable familial prevention. The 3' region of the APC gene is still the best possible location for such families. Similarly, acolonoscopy must be performed in the case of a desmoids tumor, even when it is sporadic, for detecting FAP and its risk of colorectal cancer. More information may lead to a better understanding of the molecular pathogenesis of desmoids tumors and can provide insights into the structure-function relationships of the APC protein.

Acknowledgement

The authors would like to thank Seyed Muhammed Hussein Mousavinasab for his sincere cooperation in editing this text. This study was supported by the Cancer Research Center of Tehran University of Medical Sciences.

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