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# A FAMILY WITH TWO CONSECUTIVE NONSENSE MUTATIONS IN *BMPR1A* CAUSING JUVENILE POLYPOSIS

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## Abstract

We describe a novel germline mutation in *BMPR1A* in a family with Juvenile Polyposis and colon cancer. This mutation is that of two consecutive substitutions (735-6 TG>AT) which cause two nonsense mutations (Y245X, G246X), inherited in an autosomal dominant fashion, on one parental chromosome. This mutation caused protein truncation, and represents a unique case of consecutive nonsense mutations in human disease.

### Keywords

Juvenile Polyposis; nonsense mutations; *BMPR1A*

## Introduction

Juvenile Polyposis (JP; OMIM 174900) is an autosomal dominant condition where affected individuals develop hamartomatous polyps of the colon, rectum, and less frequently, the stomach[1]. JP patients are at significant risk for developing GI cancer[2,3], and some may also have hereditary hemorrhagic telangiectasia[4,5]. Two predisposing genes have been identified in JP, *SMAD4* from 18q21[6,7], and *BMPR1A* from 10q22-23[8]. Both of these genes are members of the transforming growth factor beta superfamily, and mutations of the coding sequence of each gene have been found in approximately 20% of JP cases[9]. About half of the germline mutations thus far described have been microdeletions, and the other half substitutions. In this report, we describe the novel finding of two consecutive substitutions leading to two consecutive nonsense codons in a family with JP.

## Clinical Report

The proband (II-3, Figure 1) developed lower GI bleeding at age 5, and on sigmoidoscopy, 6 hamartomatous polyps were removed. Over the following 18 years of surveillance, 107 polyps

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were removed, and all were hamartomatous without adenomatous features. He was found to have gastric polyps at age 21. The proband's mother had a negative colonoscopy at age 42 and was still healthy at age 59 and without symptoms. His father was deemed to be healthy, but died at age 66, reportedly without polyps. The proband's paternal grandmother died of colorectal cancer at age 66. His brother had colonoscopy and an upper GI series at age 18, which were negative.

The family was lost to follow-up from 1989-1997. The proband was next seen at age 31, when he presented with jaundice, weight loss, and a bowel obstruction. At surgery, he was found to have colon cancer with liver metastases, and he died 3 months later. His older brother (II-2) resurfaced at about this time for surveillance, and was found to have numerous polyps in the colon, 3 of which had foci of adenocarcinoma. He underwent a left hemicolectomy, and has been polyp free since then. His two children (III-1, III-2) had no polyps found at colonoscopy at ages 20 and 21, respectively. The proband's daughter was 9 years of age at last follow-up, and had not had colonoscopy.

## Results and Discussion

Blood samples were obtained from individuals II-2, III-1, III-2, and III-3. DNA was extracted, and individual II-2 was sequenced for all exons and intron-exon boundaries of *SMAD4* and *BMPR1A*. Two substitutions were found in consecutive nucleotides of exon 7 of *BMPR1A* (Figure 2a), 735-6 TG>AT. Interestingly, each of these substitutions would change the corresponding amino acid into a stop codon (Y245X, G246X). Individual III-1 also had this mutation (and had no known polyps at age 20), as did III-3 (who was 9 years old at last follow-up, and had not had colonoscopy). Individual III-2 did not have the mutation and had negative colonoscopic screening at age 21 years.

mRNA was extracted from patient II-2, converted to cDNA by reverse-transcriptase PCR (using primers GGATCCTAATACGACTCACTATAGGGAGCCACCATGCCTCAGCTATACATTTAC and ATGGGTTAACCATCAGATTTTTACATCTTGGGATTCAACCATCTTGGC), and subcloned into a plasmid vector. After transformation, different recombinant clones were sequenced in order to identify both the wild-type and mutated sequences. Both substitutions were confirmed to be on the same mutant allele. Wild-type clones were 10 fold more abundant than those with mutations, suggesting a possible issue with RNA stability of the mutant sequence. Protein was synthesized using a coupled reticulocyte lysate system kit (Promega TNT). The results from SDS-PAGE revealed a truncated protein of 27 kDa corresponding to the mutant clone and the expected size of 60 kDa from the wild-type clone (Figure 2b).

There have been several reports of two consecutive substitutions and missense mutations in human disease genes. Van Belzen et al. described a Dutch family with ataxia telangiectasia (autosomal recessive) where an affected brother and sister were both homozygous for 2 consecutive substitutions in exon 55 of the *ATM* gene, leading to 2 consecutive missense mutations (D2625E/A2626P)[10]. Mullan et al. found substitutions in 2 families with early onset Alzheimer's disease that created missense mutations at 2 contiguous codons of the β-amyloid precursor protein (K670N, M671L)[11]. Another example of 2 missense mutations in a human gene was reported by Hassan et al. in the hemoglobin C gene in three affected family members, but these were not contiguous (E6V and P58R)[12].

There have also been several reports of nonsense mutations followed by missense mutations. Longy et al. described consecutive substitutions of *PTEN* in a Bannaya-Riley-Ruvalcaba syndrome patient (1735-6TA>AT), which led to a stop codon followed by missense change (Y178X, S179C)[13]. Kawabe et al. found a homozygous mutation in the dysferlin gene

(3817-8TG>AA) in a patient with limb-girdle muscular dystrophy, which resulted in a nonsense mutation followed by a missense mutation (Y1148X, G1149R)[14]. De Rosa et al. described consecutive substitutions in *APC* (3225-6TC>AA) in a patient with classic familial adenomatous polyposis and congenital hypertrophy of the retinal pigment epithelium. This results in a nonsense followed by a missense mutation (Y1075X, P1076T)[15].

To our knowledge, there has not been a description of two consecutive substitutions leading to consecutive nonsense mutations in human disease genes. Smit et al. described a patient with cystic fibrosis and a single nucleotide insertion (2307insA) in *CFTR* that led to a frameshift in codon 726, which in turn created two consecutive stop codons at amino acids 729 and 730. The patient appeared to be homozygous for this mutation[16]. Becker et al. reported a patient with congenital ichthyosiform erythroderma, which is autosomal recessive, with 2 nonsense mutations in the transglutaminase 1 gene. Each of the child's unaffected parents was heterozygous for one of the mutations (7780C>G, Y503X and 8533C>G, S669X), and therefore the nonsense mutations in this case were on separate chromosomes, nor were they at consecutive codons[17]. In our patient, subcloning of each parental copy of *BMPR1A* revealed that both nonsense mutations were inherited on the same chromosome. Our case also contrasted with these others in that JP is an autosomal dominant disorder, and inheritance of a single deleterious allele causes the phenotype. It would therefore be difficult to establish that functionally both mutations would lead to further impairment of gene function over one or the other by itself. If the disease is related to protein truncation, then there should be no difference. However, whether two premature stop codons could result in an increased level of nonsense mediated decay is less clear. The severity of disease in this family seemed more pronounced than in many others with germline *BMPR1A* mutations, as two affected individuals developed colon cancer, and one had gastric juvenile polyps, both of which are more common in JP patients with *SMAD4* rather than *BMPR1A* mutations[18,19].

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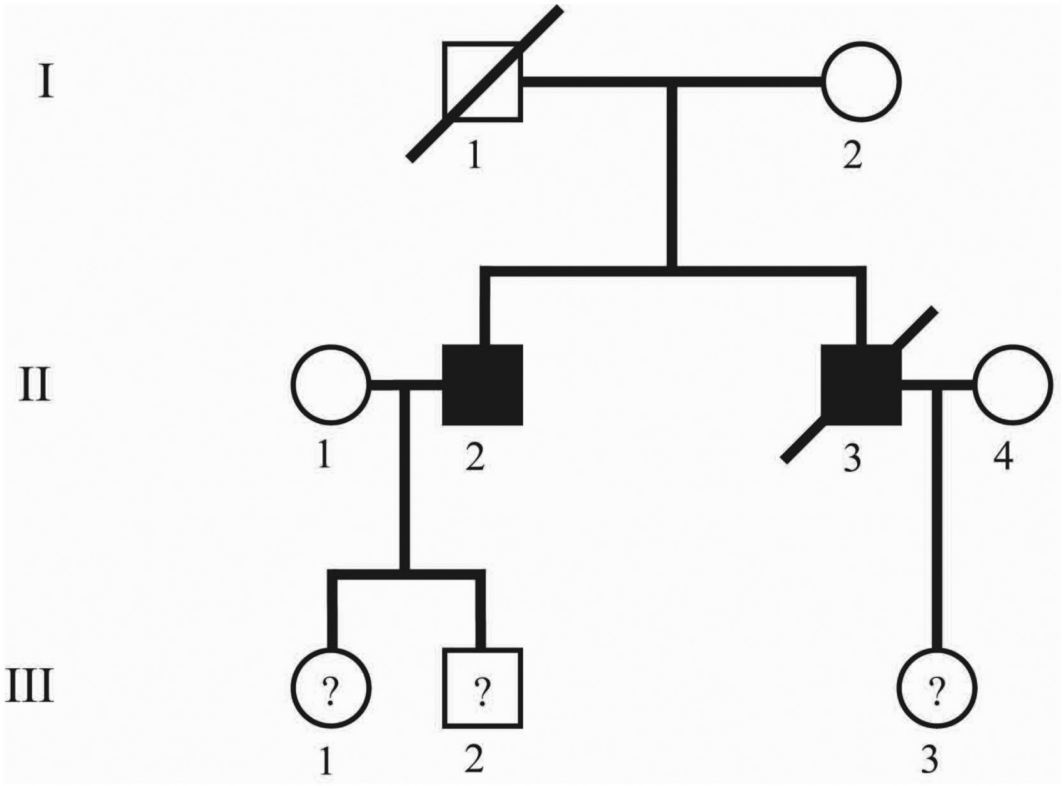
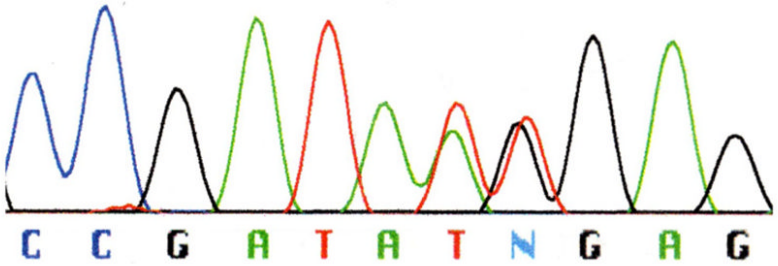



**Figure 1.**
Hungarian JP kindred. Solid shading=affected; ?=at risk.

a)

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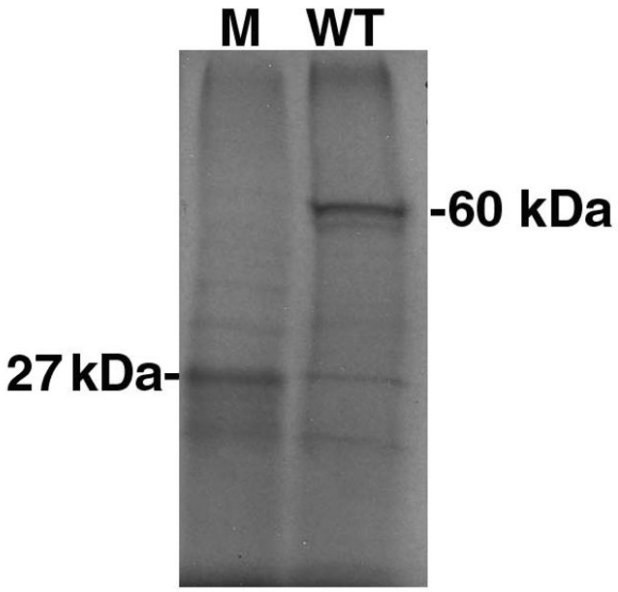


**Figure 2.**

(a) *BMPR1A* exon7 sequence of affected family member. (b) Autoradiogram of protein truncation test results, labeled using $S^{35}$-methionine, with electrophoresis through 10% SDS-PAGE. M= mutant truncated BMPR1A protein (27 kDa). WT= wild type full length BMPR1A protein (60 kDa).