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SHORT REPORT

A novel *AXIN2* germline variant associated with attenuated FAP without signs of oligondontia or ectodermal dysplasia

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Truncating mutations in the *AXIN2* gene, a key regulator of *β*-catenin degradation in the Wnt pathway, have been reported in three families with gastrointestinal adenomatous polyposis and features of ectodermal dysplasia. However, the role of *AXIN2* in familial adenomatous polyposis (FAP) syndrome is not completely understood. We performed an in-depth study of *APC* and *MUTYH*, and ruled out their implication in 23 FAP families. We then investigated the role of other genes involved in the Wnt pathway, including *AXIN2*, and identified a novel missense variant in *AXIN2* in one family with attenuated FAP. Carriers of the variant exhibited a variable number of polyps but none showed any sign of ectodermal dysplasia. We have demonstrated the pathogenicity of this novel variant by establishing its low frequency in controls as well as by LOH analysis, a segregation study, and immunofluorescent staining of AXIN2 and *β*-catenin proteins. This report expands the phenotype known to be related to *AXIN2* alterations and raises the question of whether to screen *AXIN2* in FAP cases negative for alterations in *APC* and *MUTYH*.
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INTRODUCTION

Familial Adenomatous Polyposis (FAP) is a syndrome characterized by the development of hundreds of colorectal polyps in early adolescence; it predisposes sufferers to cancer and has dominant inheritance. Attenuated FAP (AFAP) is diagnosed when patients present with less (10–99) polyps, and tends to occur 15–20 years later than for classical FAP. Mutations in the *APC* gene are responsible for the majority of FAP cases, and also explain some AFAP cases. In 2002, the implication of *MUTYH*, a BER-pathway gene, was discovered in milder cases of polyposis. Nevertheless, a considerable proportion (20–50%) of FAP and AFAP cases is not found to carry germline mutations in *APC* or *MUTYH*, and their genetic etiology remains unclear.

As *APC* has a key role in maintaining the *β-catenin* turnover in the *Wnt* pathway, other *Wnt* genes involved in this regulation might be also implicated in the syndrome. It has been suggested that *AXIN2*, the scaffold protein of the *β-catenin* destruction complex, is the main negative regulator in the pathway.[1] Little is known regarding the role of *AXIN2* in CRC, but *AXIN2* mutations have been reported in three families with gastrointestinal polyposis and ectodermal dysplasia;[2,3] the *AXIN2* truncating mutations were located in a narrow region of the gene, and the phenotype associated with them, while highly heterogeneous, always included colonic polyposis and ectodermal dysplasia.

Here we describe a family with a novel missense variant in the *AXIN2* gene, and thereby expand on the phenotype associated with alterations in this gene.

METHODS

FAP families were recruited as part of a project investigating the molecular basis of this syndrome. The project was approved by the ethics committee of the *Instituto de Salud Carlos III* and all patients provided informed consent. Twenty-three families were included in the present study because they tested negative for alterations in *MUTYH* and *APC*. The complete sequence of *MUTYH* was then studied by direct sequencing and MLPA. An extensive interrogation of *APC* involved direct sequencing of the gene, including the 1A and 1B promoters and the 3′UTR region, and assessing allelic imbalance. The Wnt genes *AXIN2* and *GSK3β* were screened for mutations using dHPLC and direct sequencing.

In order to confirm the novel origin and deleterious nature of the variant, we consulted the Human Genetic Mutation Database (HGMD), Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/) [09, 2012] and 1000 Genomes databases. LOH was assessed by PCR amplifying and sequencing a 420-bp fragment surrounding the variant in tumoral *versus* germline DNA. Immunohistochemical (IHC) staining for AXIN2, APC and *β*-catenin was carried out using the ab32197 (Abcam, Cambridge, UK), sc896 (Santa Cruz, Heidelberg, Germany) and FLEX IR702 (Dako, Barcelona, Spain) antibodies, respectively. In order to evaluate the co-localization of AXIN2 and *β*-catenin, double staining of both proteins was performed by immunofluorescence (IF) and analyzed using a SP5-WLL confocal microscope (Leica, Barcelona, Spain).

Variant frequency was assessed by genotyping a control group of 800 individuals using the KASPar SNP genotyping system (KBioscience, Berlin, Germany). Allelic discrimination was carried out using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Madrid, Spain). Evolutionary conservation of variants was evaluated as a probability using Phastcons (http://genome.cshlp.org/content/15/8/1034).

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RESULTS

A novel variant in *AXIN2* was found in one family. The *probandus* was a male who developed 10 colorectal adenomas at age of 31 years. Eleven years later, he underwent a partial colectomy to treat CRC. Subsequent colonoscopies revealed 1–2 polyps every year. His sister was diagnosed with 30 polyps and CRC at age of 36 years. Their father died from CRC at the age of 51 years. None of these cases in the family had extracolonic manifestations associated with FAP. Since the family underwent ongoing surveillance by a clinical geneticist, an oncologist and a gastroenterologist, their clinic history was extensively reviewed, but no records of ectodermal dysplasia had been reported. A subsequent dermatological examination did not identify any dental abnormalities, hair scarceness, nail development problems or skin problems, and no features of oligodontia or ectodermal dysplasia were found in the *probandus* nor in any of his four sisters (Figure 1).

The novel variant c.1387C > T; p.R463C was first found in the *probandus*. It is located in exon 5, within the *β-catenin* binding domain, and it did not appear in the public databases (HGMD, NHLBI ESP and 1000 Genomes). The Condel predictor program (http://bg.upf.edu/condel/home) classified it as deleterious with a consensus score of 0.541, although other predictor programs gave contradictory results. According to Phastcons, both the nucleotide and the domain were highly conserved through 46 vertebrates and 33 placental mammals, with a probability score of 1. It was also found to be highly conserved across 10 species of primates, the only exception being *Macaca mulata*.

Two adenomas from the *probandus* and four polyps (two adenomas, one hyperplasic polyp and one mixed polyp with both adenoma and hyperplasic features) from one sister were available for further study of LOH, as well as IHC and IF staining. LOH of the mutated allele was identified in one polyp from the *probandus*. Two polyps (one adenoma and the mixed polyp) from his sister (III-3, Figure 1) also presented LOH, whereas other adenoma (with low-grade dysplasia) had reduced amplification of one allele, and therefore

incipient LOH, but not sufficiently so to confirm LOH *per se*; her hyperplasic polyp showed no sign of LOH.

Figure 2 shows APC, AXIN2 and β-catenin IHC expression in the adenoma from the *probandus*, and in polyps from an *APC* mutation carrier and a *MUTYH* mutation carrier. Double staining by using IF suggested that in comparison with normal crypts, the adenomatous gland showed higher levels of AXIN2 and β-catenin proteins (Figure 3). Moreover, AXIN2 was accumulated in the cytoplasm, whereas β-catenin was accumulated in the membrane and the nucleus, thus confirming the lack of co-localization of these two proteins. Finally, the c.1387C > T variant was not found in any of the 800 Spanish controls. Taken together, these results point to the possible pathogenicity of the variant; thus we performed a segregation study by testing for the variant in other family members. The affected sister also carried the variant. The mother of the *probandus* (aged 82 years) was found not to carry the variant, and therefore the father was considered an obligate carrier. Two apparently asymptomatic sisters (III-3 and 4, Figure 1), aged 51 and 46 years, were both noncarriers. Both underwent periodic surveillance by colonoscopy and no polyps were diagnosed. A fourth sister (III-5), aged 43 years, was found to be a carrier of the variant. She was provided with genetic counseling and underwent a colonoscopy that showed no evidence of polyps.

DISCUSSION

The c.1387C > T variant in *AXIN2* may be related to the development of AFAP in this family. Studies carried out on tumoral tissue (LOH, IHC and co-localization assays) suggested a probable alteration of AXIN2 function. In addition, the variant appears to segregate with disease in the family, and it was not found in 800 Spanish controls nor reported in public databases, with information from over 13 000 individuals.

Although knowledge about germline *AXIN2* alterations and their associated phenotypes is limited, variants in the gene have been reported in cases with gastrointestinal neoplasia, with substantial
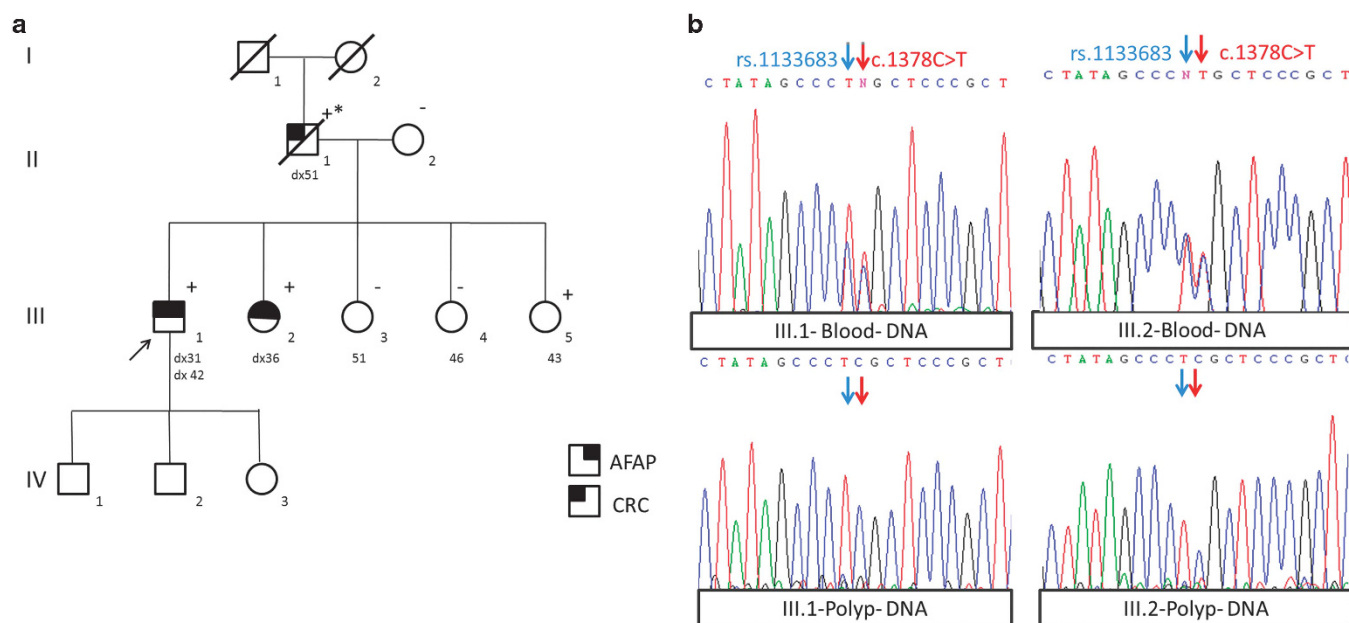

Figure 1 (**a**) Pedigree of the family. (**b**) LOH analysis in both carriers of the variant, individuals III.1 and III.2. Sequences of germline DNA from blood and polyp DNA from both affected individuals III.1 and III.2 are shown. Red arrows correspond to the variant c.1387C > T; p.R463C found in both individuals; blue arrows mark rs1133683.
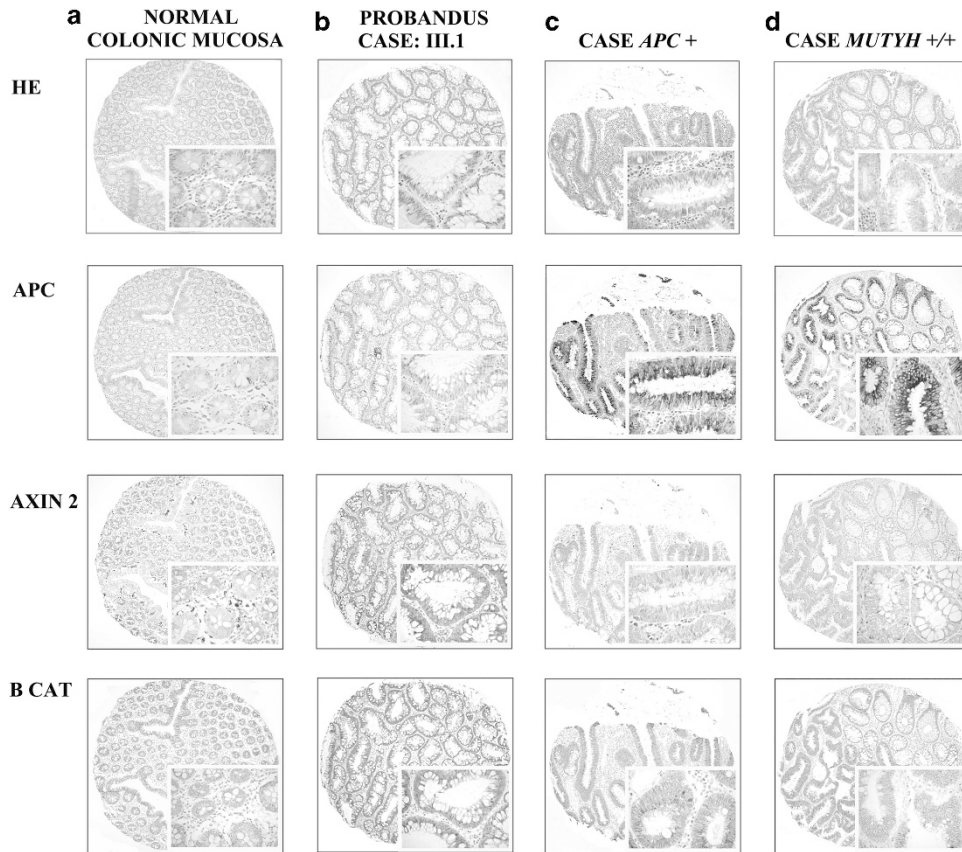
Figure 2 Immunohistochemical expression patterns of APC, *β*-catenin and AXIN2 proteins are shown in (**a**) normal colonic mucosa; (**b**) an adenomatous polyp of the *probandus* (III.1 Figure 1, carrier of the variant c.1387C>T; p.R463C); (**c**) an adenomatous polyp from an *APC c.1959-2_1960dup;p.Ile 656AlafsX20* carrier and (**d**) an adenomatous polyp from a carrier of bialellic *MUTYH* mutations *c.1187G>A;p.Gly396Asp+c.1187G>A;p.Gly396Asp*. AXIN2 overexpression was present in *probandus* compared with the *APC* and *MUTYH* mutation carriers. Moreover, APC overexpression was found in both *APC* and *MUTYH* mutation carriers but was absent in the *probandus* carrier of the c.1387C>T; p.R463C *AXIN2* variant. HE: hematoxylin and eosin staining. Staining was performed using a Tissue Microarray; the inset corresponds to a ×600 magnification. The full colour version of this figure is available at *European Journal of Human Genetics* online.
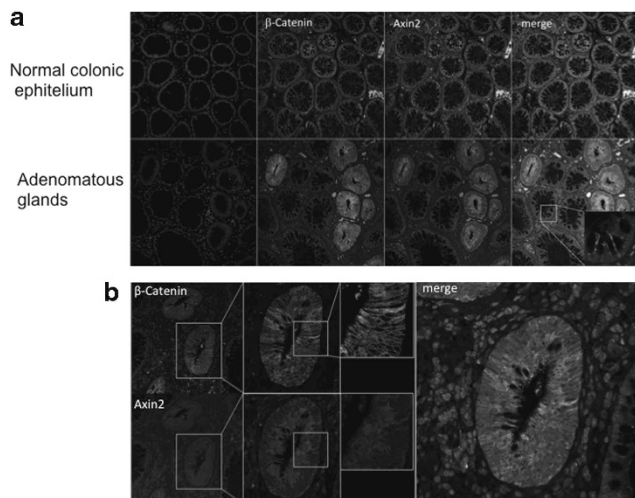

Figure 3 (**a**) AXIN2 and *β*-catenin overexpression in adenomatous polyp glands compared with paired normal colonic epithelium. The inset shows the results for a normal gland. (**b**) Lack of co-localization of AXIN2 and *β*-catenin proteins in an adenomatous polyp gland. The full colour version of this figure is available at *European Journal of Human Genetics* online.

variability in the age of onset as well as in the number and type of polyps. For example, Lammi *et al.*[2] described a family with nine carriers of a nonsense mutation in *AXIN2*; the gastrointestinal phenotype in carriers varied from 68 adenomas in a 57-year-old carrier to no adenomas in a 26-year-old and one adenoma in a 58-year-old. Even greater clinical variability was reported by Marvin *et al.*,[3] in terms of the number (from none to more than 100), type (adenomatous or hyperplastic) and location (colonic or gastric) of polyps, and the presence of neoplasias. That no polyps were found in the 43-year-old variant-carrying sister of the *probandus* in the present study is consistent with this previously observed high clinical variability.

The lack in our variant-carrying family of signs of ectodermal dysplasia and of other extracolonic manifestations frequently found in FAP and AFAP families is also remarkable. The great majority of *AXIN2* mutations have been identified in families selected because they had oligodontia, which likely introduced a selection bias into these studies. However, other conditions such as oral cleft and different types of cancer have been recently related to mutations in *AXIN2*.[4,5] Although it is possible that *AXIN2* has a role in colorectal tumor predisposition, few reports to date have tested the *AXIN2* gene in CRC/poliposis patients. Leujene *et al.*[6] studied 39 patients, one of

whom had severe oligodontia, and only two variants in *AXIN2* were found, both unlikely to be pathogenic. Peterlongo *et al.*[7] reported several *AXIN2* variants in a study of 82 patients with hereditary CRC, but none were considered pathogenic. In a study of cases negative for *APC* and *MUTYH* mutations, Renkonen *et al.*,[8] described an *AXIN2* mutation in a family with previously reported oligodontia.[2] Mongin *et al.*[9] described 38 patients with adenomas or serrated polyps in which *AXIN2* screening gave negative results. In light of these studies, it is likely that the number of polyposis patients who have undergone testing for *AXIN2* alterations is still insufficient to define the role of the gene in familial polyposis.

With the limited number of *AXIN2* mutations described to date, it is also difficult to establish a specific phenotype associated with a particular mutation. Nevertheless, all the mutations described in association with oligodontia are located in exon 7 of the gene, downstream of nucleotide 1900. The variant described here is located a substantial distance away, in exon 5. It has been described for other predisposition genes, such as *APC*, that different phenotypes may present, depending on the protein domain affected by the mutation. The existence of second and third hits in an *APC* has been previously reported, and explained using the 'just right level of β-catenin' model.[10] We have identified LOH of the mutated allele that would constitute a second hit event; taking into account the accumulation of AXIN2 protein in the cytoplasm, we speculate that a third hit event may occur in the wild-type allele, leading to a dominant-negative effect by the retained product. Therefore, further studies of larger patient series, as well as functional assays, are needed to clarify the role of *AXIN2* in FAP.

In summary, we have identified a novel missense *AXIN2* variant present in an AFAP family with no signs of oligodontia, ectodermal dysplasia or EM. This report potentially extends the phenotype caused by *AXIN2* alterations and, though the associated phenotype is still not well understood, the testing of *AXIN2* in FAP cases that are found to be negative for *APC* and *MUTYH* mutations may be an important clinical consideration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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