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Germline mutations in the β-catenin gene are not associated with the FAP phenotype without an APC mutation

EDITOR—Familial adenomatous polyposis (FAP) is generally considered a typical monogenic disease caused by germline mutations within the adenomatous polyposis coli (APC) gene. Despite applying several screening techniques, however, mutational studies world wide have failed to identify a germline mutation within the APC gene in up to 50% of all FAP cases (called APC negative below).[1 2] Intronic alterations, mutations within the regulatory regions causing altered APC gene expression, or large scale rearrangements of the APC gene could account for the failure to identify an APC mutation in some FAP families. Assuming no hot spots, however, such mutations are not likely to cause a high percentage of APC negative cases.

Fifty families which were referred to our department with the clinical diagnosis of FAP were tested for APC germline mutations. In 14 of them (28%), no APC mutation could be identified after screening the entire coding region of the gene (APC negative group) using the protein truncation test (PTT, the whole coding region), single strand conformation polymorphism analysis (SSCP, the affected PTT segment), and direct DNA sequencing (the affected SSCP exon).[3] As these families were either too small or only a limited number of family members were available, linkage analysis with respect to the APC locus could not be performed. They all fulfil the major criteria for the clinical diagnosis of FAP (table 1). We have shown previously that these families differ phenotypically from those with an APC mutation, suggesting that they might represent a distinct genetic entity.[4] In summary, the APC negative group tended to have less severe disease characteristics with significantly increased age at diagnosis, fewer colonic polyps, and fewer extracolonic manifestations, so they are similar to those with attenuated adenomatous polyposis coli (AAPC). However, all three regions of the APC gene which have been reported to correlate with AAPC[5] were analysed without detecting DNA abnormalities.

A candidate which might be involved in the locus heterogeneity in FAP is the β-catenin gene, a member of the same cellular pathway as APC. Free β-catenin is targeted for degradation by the glycogen-synthase kinase (GSK) 3β and the APC protein. An increase in the stability of free β-catenin is triggered by the Wnt signal as well as by the absence of APC protein or the presence of mutated APC protein.[6] Finally, if APC is intact, mutations within the β-catenin gene itself could also result in an increased level of free β-catenin.[7] In recent studies, somatic mutations in the β-catenin gene were found in colorectal and other types of tumours.[7–12] However, none of the studies observed a germline mutation within the β-catenin gene in tumour matched constitutional DNA.

Based on the observation that somatic mutations in the β-catenin gene can mimic mutations in the APC gene in colonic tumours, the possibility arises that, similarly, germline β-catenin mutations could mimic germline APC mutations giving rise to an FAP-like phenotype. In the present study this hypothesis was tested.

In 14 APC negative FAP families, the entire coding region (16 exons) of the β-catenin gene (CTNNB1) was screened in DNA isolated from peripheral blood. SSCP analysis with PCR primers allowing investigation of intron/exon boundaries (table 2) was applied. All but one exon were analysed in a single PCR-SSCP reaction. Owing to its length exon 9 was screened in two separate reactions. No new conformers in any of the exons were detected, suggesting that DNA alterations in the β-catenin gene did not occur. We are aware of the possibility that some mutations might have been missed; however, SSCP is one of the most reproducible techniques for mutation analysis with the reported sensitivity under optimised conditions reaching 90%.[13]

Interestingly, in previous studies, β-catenin mutations were found almost exclusively in the NH2-terminus of the gene and altered either the phosphorylation sites or a

Table 1 Phenotypes of FAP patients without an identified APC mutation

Family/ patient	*Phenotype of polyposis*	*No of polyps*	*Age at diagnosis*	*Cancer*	*EM*
18/1	Yes	>100	41	—	—
18/6	Yes	>30	13	—	—
18/101	Yes				—
18/102	Yes				—
18/103	Yes				—
18/104	Yes				—
1465/2	Yes		64	—	—
1465/7	Yes	>10	31	—	—
1465/101			64	Oesophagus/lung	—
1465/102				Stomach	—
1465/103	Yes			Colon	—
1505/1	Yes	>100	48	Rectum	—
1522/1	Yes		46	Rectum	—
1545/1	Yes		54	—	—
1546/1	Yes	>100	21	—	SIP
1551/1	Yes		47	—	—
1551/2	Yes			—	—
1551/101				Colon	—
1552/1	Yes		72	Meningioma	Desmoid
1596/1	Yes		39	—	—
1596/101	Yes		37		—
1596/102	Yes		31		—
1596/103	Yes				—
1596/104	Yes		22		—
1596/105			38	GIT	—
1596/106			50	GIT	—
1596/107			50	GIT	—
1596/108	Yes				—
1665/1	Yes		54	—	Osteoma
1666/1	Yes	>10	56	—	—
1666/101	Yes		45	Colon	—
1666/102			60	Colon	—
1666/103			27	Sarcoma	—
1705/1	Yes		48	—	—
1724/1	Yes	>100	46	Sigmoid	—
1724/2	Yes	<100	44	—	—
1724/101			40	GIT	—
1724/102	Yes	>100	63	Rectum	—
1724/103			41	GIT	—
1724/104			45	CSU	—
1736/1	Yes		46	Colon	—

EM - extracolonic manifestations, SIP - small intestinal polyps, GIT - gastrointestinal tract, CSU - cancer site unknown, blank - data unknown.

Table 2 PCR primers for SSCP analysis of 16 exons of the β catenin gene

Exon	*Forward primer 5'-3'*	*Reverse primer 5'-3'*	*Length of PCR product (bp)*
1	1. 1 attttaagcctctcggtct	1. 2 aagcacctcaggggaaca	177
2	2. 1 ttagggtatttgaagtata	2. 2 acaaaccttgagtagccatt	71
3	3. 1 atagctgatttgatggagtt	3. 2 tcttaccagctacttgttctt	238
4	4. 1 cagatattgatggacagtat	4. 2 ttacctggtcctcgtcattt	261
5	5. 1 tacaggtggtggttaataag	5. 2 ttcttacccaagcattttca	251
6	6. 1 cagttcaccagtggattct	6. 2 ttaccttgctttcttggttg	209
7	7. 1 tagctcatcatactggctagt	7. 2 ttaccagcttctacaatag	152
8	8. 1 taggtggaatgcaagcttta	8. 2 ttacctgtttagttgcagca	111
9a	9a. 1 ataggaagggatggaaggt	9a. 2 aggctcagtgatgtctt	208
9b	9b. 1 tcttgtgcgtactgtcctt	9b. 2 acaatttacctttatcaga	196
10	10. 1 tcaggctactgttggattgatt	10. 2 taccacaaattgctgctgt	166
11	11. 1 ttcttttggcaggagg	11. 2 acatacctgcacaaacaat	138
12	12. 1 tccttagctgctttattct	12. 2 acttacccacaccttcatt	164
13	13. 1 tgtctcttagcgacatat	13. 2 tacctcattccaagccatt	135
14	14. 1 tagactgctgatcttggactt	14. 2 ataccatcctggcgata	68
15	15. 1 tctagatcctagctatcgttc	15. 2 ttacctaaaggatgattta	230
16a	16a. 1 agctgtattgtctgaactt	16a. 2 tttaacaagcaaggctag	264
16b	16b. 1 cacaagatggaatttagcaa	16b. 2 ccatttttctatttgtctatt	325
16c	16c. 1 aagtgtaacaattgtgtag	16c. 2 tgacacaatcgaatgaat	306

domain responsible for binding to the APC protein.[7–12] Most frequently affected codons were localised in exon 3 (33, 44, and 45). Therefore, we additionally analysed exon 3 by direct DNA sequencing using the same primers as for SSCP, the Thermosequenase labelled primer cycle sequencing kit (Amersham), and an automated DNA sequencer (LI-COR) without identifying any changes in the nucleotide sequence.

As there were no differences detected in the coding region, β-catenin gene expression in patients' lymphocytes was examined using reverse transcription PCR. PCR amplification of cDNA was performed in two separate PCR reactions using PCR primer pairs 3.1/6.2 and 6.1/13.2, as listed in table 2. This showed that in all persons tested the expected β-catenin transcript was present and of expected size, suggesting that differential splicing did not occur. However, as our experiments regarding β-catenin expression were performed on lymphocytes, differential expression in colonic or other tissues affected in FAP, which were not available for our study, cannot be excluded.

Taken together, using a combination of several techniques for the mutational analysis, in our group of 14 FAP APC negative families, no hereditary alterations were identified in the β-catenin coding sequence or gene expression, suggesting that β-catenin germline mutations do not account for APC negative FAP cases. Even though these results need confirmation in a larger sample of APC negative FAP families, they indicate that β-catenin might play a different role in the pathogenesis of hereditary colon carcinoma compared to sporadic colorectal cancer.

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Simpson-Golabi-Behmel syndrome and attention deficit hyperactivity disorder in two brothers

EDITOR—Simpson-Golabi-Behmel syndrome (SGBS, MIM 312870) is an X linked condition characterised by pre- and postnatal overgrowth, coarse facial appearance, large mouth, predisposition to embryonic neoplasia,[1] and a variety of visceral and skeletal abnormalities. Psychomotor development in the syndrome is extremely variable, ranging from normal intelligence,[2] to moderate impairment, to severe impairment evident from birth.[3 4] We report the cases of two male sibs with normal psychomotor development, diagnosed at 6 and 7 years of age with SGBS, who manifest significant behavioural disturbances consistent with a diagnosis of attention deficit hyperactivity disorder (ADHD). This is the first report of an association between SGBS and a specific behavioural phenotype (ADHD).

Case 1, the older of the two boys, was the first born to non-consanguineous, healthy, white parents. The pregnancy was complicated at 36 weeks by polyhydramnios and pregnancy induced hypertension. An ultrasound performed at this time discovered a left sided diaphragmatic hernia. No other fetal abnormalities were reported. Labour was induced at 39 weeks and the child was delivered by forceps assisted vaginal delivery. Birth weight was 4400 g (well above the 97th centile) and immediate transfer for stabilisation and surgical repair of the diaphragmatic hernia was undertaken. Other birth indices were not recorded. The primary surgical repair was successful but was complicated by a left pneumothorax on day 4 of life. This complication was successfully managed and the subsequent postsurgical course was uneventful. The child was discharged from hospital aged 14 days.

At 5 months bilateral inguinal hernias were noted and surgically repaired. From 6 months onwards the child was noted to be extremely unsettled, seldom sleeping for more than two hours at a time. From 10 months onwards he began to wake screaming several times a night. His behaviour became increasingly unpredictable and aggressive over the next year, with high levels of activity and very poor attention span reported. He was assessed at 3 years 11 months by a mutidisciplinary team consisting of a paediatrician, speech pathologist, clinical psychologist, child psychiatric nurse, and occupational therapist. The McCarthy Scales of Children's Abilities was administered and he performed at a 4 year old level on the verbal (score 46, mean score for age 50, SD 10), perceptual performance (score 50), and quantitative (score 53) scales and at a 3.5 year old level on the memory scale (score 44). The general cognitive index was age appropriate (score 96, mean for age 100, SD 16) and his overall cognitive age was placed at 4 years ± 6 months. He also showed relative strengths in conceptual grouping tests (performed at the 6.5 year old level), counting and sorting (6 year old level), and opposite analogies (5.5 year old level). These data indicated sound verbal concept formation skills, arithmetic ability, and a sound capacity for abstraction. Areas of greatest difficulty were in Verbal Memory tests I and II where he performed at the 3.5 year old level. Short term memory and attention span were noted to be extremely poor in relation to other abilities documented above. On Word Knowledge tests I and II he performed at the 3 year old level and he was noted to dis-