Original Paper

European Journal of Human Genetics

Eur J Hum Genet 1997;5:43–49

Received: July 2, 1996
Revision received: December 16, 1996
Accepted: December 19, 1996

Association of Extracolonic Manifestations of Familial Adenomatous Polyposis with Acetylation Phenotype in a Large FAP Kindred

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Abstract

Familial adenomatous polyposis coli (FAP) has been shown to be associated with germline mutations of the adenomatous polyposis gene (APC) on chromosome 5. Extra-colonic manifestations also occur in FAP and include desmoid tumors, epidermoid cysts and osteomas. The combination of FAP with extracolonic symptoms is commonly referred to as Gardner's sydrome. It remains difficult, however, to predict which patients may have a propensity to develop extracolonic manifestations. The rapid acetylation phenotype is believed to be associated with an increased likelihood of sporadic colorectal cancer, whereas the slow acetylation phenotype is recognized as a predisposing factor for bladder cancer. The slow acetylation phenotype is caused by mutant alleles of the cytosolic enzyme N-acetyltransferase (NAT2). In this study, we determined the NAT2 genotype in members of one large FAP family and three smaller ones all of which had been shown to harbor the same germline APC gene mutation. We observed a significant correlation between slow acetylation genotypes and extracolonic manifestations of the disease. Rapid acetylation genotypes were not overrepresented in colorectal cancer cases in this family as compared to the frequency of this genotype in the normal Caucasian population.

Key Words
Familial polyposis coli
Extracolonic disease
N-acetyl transferase

Introduction

Familial adenomatous polyposis (FAP) is an autosomal-dominantly inherited disease affecting approximately 1 in 10,000 people [1] and is characterized by the development of hundreds to thousands of colorectal polyps [2], which if left untreated would almost certainly develop into colorectal cancer [3]. In association with FAP there exists a more severe form of the disease, otherwise known as Gardner's syndrome, which includes extracolonic manifestations such as osteomas, epidermoid cysts, small intestinal carcinomas, gastric polyps, thyroid carcinomas and dental abnormalities [4]. However, a precise definition of this entity is lacking. Common to both forms of the

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1018–4813/97/0051–0043$12.00/0

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disease is the increased likelihood to develop desmoid tumors which appear to be a significant side effect after surgery [5].

The FAP locus was identified after the observation of a constitutional interstitial deletion on chromosome 5q in a mentally retarded patient with FAP and a desmoid tumor [6] followed by linkage analysis to the 5q21-22 locus [7]. More recently, the gene involved in the inheritance of FAP has been sequenced and is known as the APC gene [8, 9]. Many APC germline mutations have been found in FAP patients and in presymptomatic carriers [10–15]. Of particular note is that identical APC gene mutations have been identified in unrelated and related FAP families presenting with or without extracolonic manifestations [16, 17]. The most likely explanation is the presence of a modifying gene that is able to influence disease expression. Support for the existence of a modifying locus comes from studies using the naturally occurring multiple intestinal neoplasia *(Min)* mouse model [18]. More recently, a candidate modifier gene has been identified in the mouse, namely secretory phospholipase A_2 [19]. This gene does not, however, appear to be associated with phenotypic modification in man as in both FAP families and sporadic colorectal cancer patients no association has been identified [20, 21].

It has been recognized that acetylation catalyzed by the cytosolic enzyme N-acetyltransferase (NAT) is an important process in the activation of arylamine carcinogens and that there exists genetic variation within the population, such that there are rapid and slow acetylators [22]. The two polymorphic isozymes NAT1 and NAT2 perform the function of arylamine N-acetylation and O-acetylation of hydroxyamines to yield acetoxy derivatives [23, 24], respectively. It has been shown that only the isoenzyme NAT2 is responsible for the acetylation polymorphism [25]. Six point mutations of the NAT2 gene on chromosome 8 have been identified which either occur as single mutations or in combination. They produce over 10 mutant alleles of the NAT2 gene. Three alleles (M1, M2, M3) account for over 95% of the slow acetylation phenotype in most populations [26–29].

The frequencies of rapid and slow acetylators vary remarkably in different ethnic populations. In Europe and North America there are about 40–70% slow acetylators, compared to only 10–20% in Japan and China [30].

Two studies have shown that sporadic colorectal cancer (CRC) tends to be associated with a rapid acetylator phenotype [31–33] which is believed to be a predisposing factor in the development of the disease. The slow acetylator phenotype is overrepresented in patients with bladder cancer that were exposed to amine carcinogens [34–36].

In this study we examined one large kindred with FAP which included family members with extracolonic manifestations and three smaller families. Previously, we have shown that all affected members of the large kindred have inherited the same germline mutation in the APC gene [17]. Three additional smaller families not known to be related to the large family at the time of the analysis were also included in the study as they too harbored the same APC germline mutation. Subsequent haplotype and pedigree analysis of all four families has since revealed that they are related sharing a common ancestor in the early 18th century. Genotyping analysis of the NAT2 gene revealed that persons classified for this study as suffering from either extra-colonic disease alone or in combination with colonic disease, appeared to correlate with a particular acetylation status. In contrast, no difference between the frequency of slow or rapid acetylators in the subgroup suffering from colonic disease alone and the general population was observed.

Materials and Methods

Patients

Symptomatic patients were first ascertained at clinical presentation, thereafter relatives were contacted through their family doctor after the index patients had filled out a questionnaire pertaining to FAP in their family. The clinical diagnosis of FAP was based on colonoscopy. When only colonic symptoms were evident they were designated as FAP patients, whereas those patients who had any other signs before or after colonic disease were referred to for this analysis as having extracolonic disease plus or minus FAP.

DNA Isolation

Genomic DNA was isolated from EDTA blood according to the method described by Miller et al. [37].

Detection of the NAT2 Genotype by PCR Amplification

The specific amplification of the NAT2 alleles was carried out in separate PCR reactions for the wild-type and mutant alleles of M1, M2 and M3. The primers and amplification procedures used have been described previously [26]. Persons that were homozygous or heterozygous for a wild-type allele were designated as having a rapid acetylation genotype. Those being homozygous for one or compound heterozygous for two mutated NAT2 alleles were designated as slow acetylators. In a single case the genotype of one individual was deduced by pedigree analysis of the first-degree relatives and that of the spouse. Precise studies have shown that the phenotype can be predicted by these techniques in over 90% of the cases [38].

Statistical Analysis

Fisher's exact test and a binomial test were used to determine differences between FAP patients and patients with extracolonic disease ± FAP.

Results

A detailed pedigree of the very large family used for this investigation has been reported previously [17]. A condensed version of the family is shown in figure 1 which indicates only the most important branches of the family, age of diagnosis and acetylation genotype. Every affected person in all four families harbored the same germline APC mutation. Table 1 provides a brief profile of disease phenotypes and NAT2 genotypes. Asymptomatic APC gene mutation carriers were excluded from any calculation as it is impossible to predict what disease phenotype they will eventually express.

From a total of 127 persons, 52% were rapid acetylators and 48% were slow acetylators.

A comparison of NAT2 genotypes between all affected APC gene carriers and healthy controls revealed no deviation from independence (table 2) implying that apparent differences between the frequencies of the slow/rapid genotypes within the two groups were likely due to chance alone. If the gene carrier group was further subdivided into patients with FAP alone and patients with extracolonic disease ± FAP, several differences are observed. Between the control group and FAP no significant difference was seen ($p = 0.172$). When the extracolonic disease group was compared to the control group a significant difference between the presence of slow and fast acetylators was observed ($p = 0.022$, data shown in table 3). Finally, comparing the extracolonic disease group to the FAP group, a highly significant difference was observed ($p = 0.004$) between the two groups in that slow acetylation appeared to be almost exclusively associated with extracolonic disease. In summary, the extracolonic disease group has a markedly different acetylation genotype than either the FAP or healthy controls.

One problem with the above analysis is that it assumes that the genotype of the patients under investigation are independent of one another. Since it is not clear how deviations from independence will affect the analysis or interpretation of the results, other tests that take this into account need to be employed. In an attempt to overcome these shortcomings, we have compared the NAT2 genotypes of patients who are the offspring of matings between compound heterozygotes or homozygotes for M1, M2,

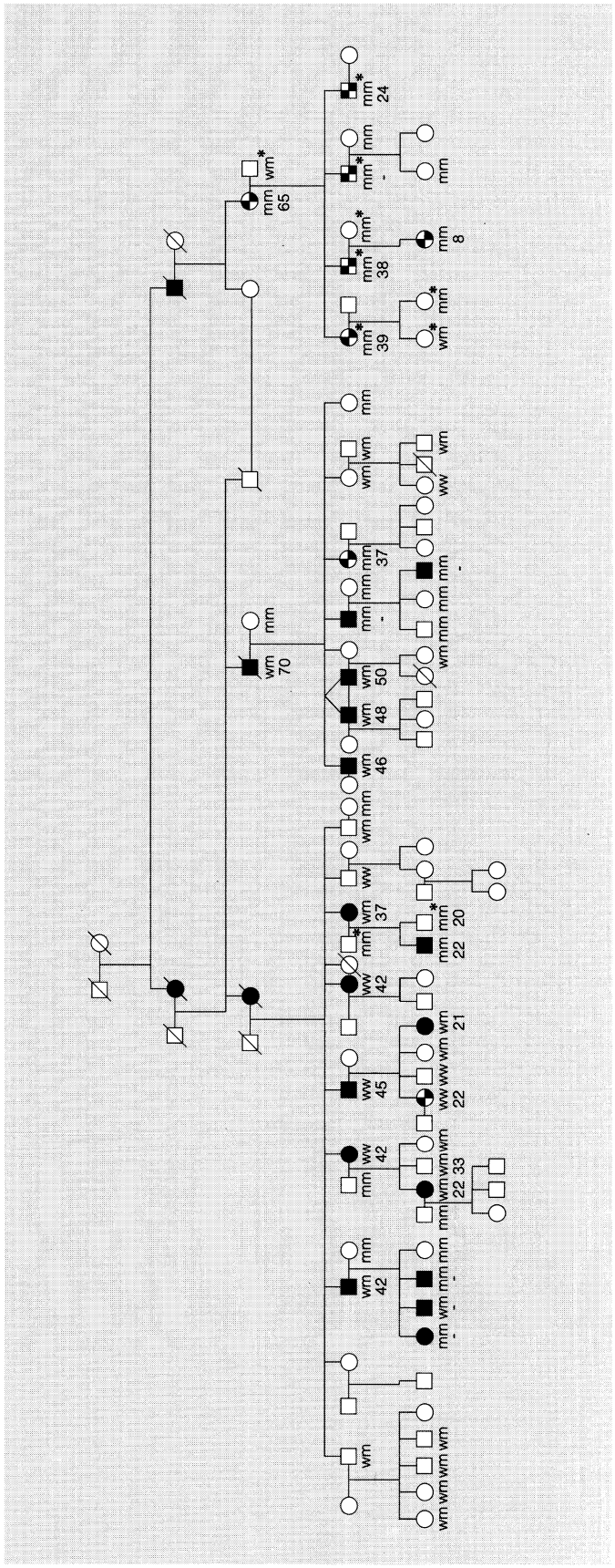

Fig. 1. Abridged version of the large kindred indicating age of onset and genotype analysis in persons shown. Checkered symbols denote EC ± FAP phenotype and black symbols denote FAP.

Table 1. Description of disease presentation and genotype analysis for NAT2 in APC gene carriers all harboring the same mutation

Patient	Sex	Extracolonic disease	Colonic disease[1]	Genotypes
Family 1				
1	M	–	+	Wt/M1 (rapid)
2	F	desmoids	+	M1/M1 (slow)
3	M	–	+	Wt/M1 (rapid)
4	M	–	+	M1/M1 (slow)
5	F	–	+	M1/M1 (slow)
6	F	–	+	Wt/Wt (rapid)
7	F	–	+	Wt/M1 (rapid)
8	M	–	+	Wt/M1 (rapid)
9	M	–	+	Wt/Wt (rapid)
10	F	fibroma	+	Wt/Wt (rapid)
11	F	–	+	Wt/M1 (rapid)
12	F	–	+	Wt/Wt (rapid)
13	F	–	+	Wt/M1 (rapid)
14	M	–	+	Wt/M1 (rapid)
15	M	–	+	M1/M2 (slow)
16	M	–	+	Wt/M1 (rapid)
17	M	–	+	Wt/M1 (rapid)
18	M	–	+	Wt/M1 (rapid)
19	M	–	+	Wt/M1 (rapid)
20	M	–	+	M1/M1 (slow)
21	F	desmoid	+	M1/M1 (slow)
22	M	gastric polyposis	+	M1/M1 (slow)
23	M	–	+	Wt/M1 (rapid)
24	M	–	+	Wt/M2 (rapid)
25	F	–	+	Wt/M2 (rapid)
26	M	–	+	M1/M2 (slow)
27	M	–	+	M1/M2 (slow)
28	M	–	+	M1/M2 (slow)
29	M	osteoma	–	M1/M1 (slow)
30	M	–	+	M1/M1 (slow)
31	F	desmoid	+	M1/M1 (slow)
32	F	desmoid	+	M1/M1 (slow)
33	F	gastric polyposis	+	M1/M2 (slow)
34	F	oesteoma	–	M1/M1 (slow)
35	M	gastric polyp	+	M1/M2 (slow)
36	M	gastric polyposis	+	M1/M2 (slow)
Family 2				
1	F	desmoid	+	M2/M2 (slow)
Family 3				
1	M	gastric polyposis	+	M2/M2 (slow)
Family 4				
1	M	desmoid	+	M1/wt (rapid)
2	M	desmoid	+	M1/M1 (slow)
3	M	–	+	M1/M3 (slow)

[1] The data available for this study with respect to colonic disease characteristics (i.e. how many adenomas were present in each patient) are unreliable, therefore we only designated here whether a patient had colonic disease at the time of writing or not.

Table 2. Distribution of rapid and slow acetylators genotypes from all tested families

	Total	Rapid acetylators		Slow acetylators	
		n	%	n	%
APC-gene mutation carrier	44	21	48	23	52
Healthy persons	83	46	55	37	45
Total	127	67	53	60	47

No statistical difference between mutation carriers and healthy persons as judged by Fisher's exact test.

Table 3. Comparison between acetylation genotype in affected family members and their healthy relatives

	Total	Rapid acetylators		Slow acetylators	
		n	%	n	%
FAP	26	18	69	8	31
EC disease ± FAP	15	3	20	12	80
Healthy	83	46	55	37	45

A statistical difference observed between the EC and the control group ($p = 0.022$) and between the EC and the FAP group ($p = 0.004$) using Fisher's exact test with respect to acetylation status. EC = Extracolonic.

M3 and heterozygotes for W (table 4) and therefore have a 50% probability of inheriting the W allele. Although the numbers are small, it is noticeable that all the extracolonic disease patients were slow acetylators ($p = 0.03$ for a binomial test of deviation from 50:50 expectation), whereas the FAP patients conformed to expected values from such crosses.

Together, the results imply that there is a relationship between slow acetylation and the likelihood of extracolonic disease development due to the paucity of rapid acetylators in the extracolonic disease subgroup.

Discussion

Of considerable interest to the clinician would be the possibility of identifying an additional genetic or environmental factor that may predispose a person already har-

Table 4. Observed versus expected number of patients with slow and rapid acetylation genotype among progeny of crosses between persons homozygous for M and heterozygous for W alleles

	WM	MM
FAP	7 (6)	5 (6)
EC ± FAP	0 (2.5)	5 (2.5)

Expected values shown in parentheses. M denotes either M1, M2 or M3, all isoforms result in slow acetylation and are recessive. W denotes rapid acetylation and is dominant in any combination with M1, 2, or 3. Among EC ± FAP patients, proportions are different from mendelian expectations (binomial test $p = 0.03$).

boring an APC mutation to the development of extracolonic disease [39, 40]. In this report we have attempted to correlate the acetylation genotype to the likelihood of extracolonic disease development.

The existence of families that carry the same germline mutation in the APC gene yet present with markedly different phenotypes is indicative of other influences affecting disease expression. Strong candidate genes that may modify disease expression include enzymes that are associated with carcinogen metabolism, such as NAT2. In this report four families are presented where affected persons all carry the same germline mutation, yet present with markedly varied disease phenotypes. Therefore, differences in disease expression cannot be attributed to mutations occurring at different sites within the APC gene which has previously been shown in some cases to be correlated with disease presentation. Together this implies that the most likely cause of disease diversity is the influence of one or more modifying genes. One modifier gene, phospholipase A_2, has recently been described which appears in mice to alter the number of polyps and age of onset of colonic disease [19]. Recent evidence indicates, however, that this gene is not associated with phenotypic variation in man [20, 21]. It cannot be excluded, however, that the diversity of phenotype seen in families harboring truncating 3′ APC germline mutations is due to the instability of the resulting prematurely terminated protein product which results in a null APC allele. The presence of a null APC allele implies that APC carcinogenesis is driven via haplo insufficiency and is not due to the effects of a protein exhibiting only partial function [41]. This effect may not be seen in families where the more common APC mutations have been identified as these would lead to a putative dominant negative effect and hence could negate any influence brought about by a modifying factor. Thus it is possible that the NAT2 slow acetylator genotype represents a modifier of null APC mutations and not necessarily of the more common APC mutations that lead to a stable truncated protein.

In an attempt to determine if other factors were involved in the expression of extracolonic manifestations of FAP, a difference in the acetylation capacity of persons carrying a specific germline mutation in the APC gene was considered. Previously, it has been shown that there is an apparent association between rapid acetylation and sporadic colorectal cancer [31–33]. Conversely, the slow acetylator phenotype is overrepresented in patients with bladder cancer [34–36], indicating that the two different environments may be important with respect to disease expression. Bladder cancer has not been reported in any of the patients used in this investigation.

In the present study, we subdivided all clinically verified patients with an APC gene mutation into either colorectal cancer alone (FAP) or colorectal cancer in association with extracolonic manifestations that occurred before or after colonic disease. The original description of FAP includes desmoid tumors. Desmoid tumors are relatively common complications in FAP patients after colonic surgery and are often associated with FAP and are not necessarily present in persons who exhibit other extracolonic symptoms [5]. For the purposes of the current study patients who had developed desmoid tumors after surgery were classified as having extracolonic disease as we wanted to determine if patients who developed any extracolonic lesions before or after colorectal disease carried an additional predisposition. Interestingly, most patients within the extracolonic disease group presented initially with an extracolonic manifestation (such as osteomas or epidermal cysts) at or before colonoscopy.

From this family study, we found that the slow acetylation phenotype appears to be associated with the development of extracolonic disease in persons predisposed to the development of polyposis coli. The lack of association between rapid acetylators and persons expressing only FAP is not surprising. The most likely explanation for this observation is that persons who carry a germline mutation of the APC gene are at such an increased risk of developing colorectal cancer that acetylation is unlikely to play a significant role in CRC development.

The question arises as to how acetylation status plays a role in the expression of extracolonic disease. It has been

documented that there is an association of rapid acetylation and colorectal cancer [31–33] and that the predisposition towards colorectal cancer is due to the rapid acetylation of aryl amines in the liver and gut such that few if any of these carcinogenic compounds reach other organs. An intriguing aspect is the association of slow acetylation and bladder cancer presumably due to a higher concentration of aryl amines in the bladder which could act on the bladder mucosa. Similarly, it could be expected that in slow acetylators, aryl amines may be present at a higher concentration within the body such that they may affect sensitive tissues and predispose an APC gene carrier to extracolonic disease. As the colon has been shown to be a major site of acetylation in comparison with the liver [30], it is possible that after colectomy there is a reduction in acetylation capacity. Alternatively, heterogeneity of the acetylation phenotype may exist within each individual thus leading to different clinical outcomes. One aspect that could not be addressed in this study concerns those slow acetylators who had thus far only presented with colorectal disease. Will they develop other symptoms associated with polyposis coli? Furthermore, it is possible that the rapid acetylators develop extracolonic disease as they get older and thus eliminate any differences between rapid and slow acetylators with respect to extracolonic disease development. The evaluation of age adjusted phenotype classes would better define the role of NAT2 in extracolonic disease development. These questions can only be answered by continued surveillance and more rigorous screening for other manifestations of the disease.

In conclusion, we have demonstrated in one large family and several smaller ones all carrying the same APC germline mutation an association of slow acetylation genotype and extracolonic disease. Due to the paucity of cases it is not possible to proceed much further with this analysis in our study population. Furthermore, it cannot be ruled out that the association described here is peculiar to the APC gene mutation found in these families. Therefore, to confirm that slow acetylators may be associated with an increased risk of extracolonic disease, additional studies are required using as many clinically and genetically verified cases as possible. This we believe could only be performed with a large multicenter study.

Acknowledgements

This work is supported in part by grants from the Swiss National Foundation No. 3200-042558.94 and the Swiss Cancer League AKT 78.

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