Analysis of genetic and phenotypic heterogeneity in juvenile polyposis

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Abstract

***Background*—Juvenile polyposis syndrome (JPS) is characterised by gastrointestinal (GI) hamartomatous polyposis and an increased risk of GI malignancy. Juvenile polyps also occur in the Cowden (CS), Bannayan-Ruvalcaba-Riley (BRRS) and Gorlin (GS) syndromes. Diagnosing JPS can be problematic because it relies on exclusion of CS, BRRS, and GS. Germline mutations in the *PTCH*, *PTEN* and *DPC4* (*SMAD4)* genes can cause GS, CS/BRRS, and JPS, respectively.**

***Aims*—To examine the contribution of mutations in *PTCH*, *PTEN*, and *DPC4* (*SMAD4)* to JPS.**

***Methods*—Forty seven individuals from 15 families and nine apparently sporadic cases with JPS were screened for germline mutations in *DPC4*, *PTEN*, and *PTCH*.**

***Results*—No patient had a mutation in *PTEN* or *PTCH*. Five different germline mutations were detected in *DPC4*; three of these were deletions, one a single base substitution creating a stop codon, and one a missense change. None of these patients had distinguishing clinical features.**

***Conclusions*—Mutations in *PTEN* and *PTCH* are unlikely to cause juvenile polyposis in the absence of clinical features indicative of CS, BRRS, or GS. A proportion of JPS patients harbour *DPC4* mutations (21% in this study) but there remains uncharacterised genetic heterogeneity in JPS.**

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Juvenile polyposis (JPS; MIM 174 900) is a rare autosomal dominant condition characterised by hamartomatous polyps, usually within the colon but occasionally arising in the stomach and small bowel.[1] These polyps are typified by a predominant stroma, cystic spaces, and an abundant lamina propria lacking smooth muscle, so distinguishing them from Peutz-Jeghers polyps. Unlike solitary juvenile polyps which may affect up to 2% of children and adolescents and have little or no malignant potential, JPS patients have an increased risk of gastrointestinal malignancy.[2 3] JPS may occur in association with arteriovenous malformations (MIM 175 050) but it is not clear if this represents a distinct syndrome.

Juvenile polyps also occur as a manifestation of the dominantly transmitted familial cancer syndromes: Cowden syndrome (CS; MIM 158 350) characterised by multiple hamartomas, macrocephaly, trichilemmomas, and a high risk of benign and malignant neoplasms of the thyroid, breast, uterus and skin; Bannayan-Ruvalcaba-Riley syndrome (Bannayan-Zonana syndrome, BRRS, BZS; MIM 153 480) characterised by mental retardation, macrocephaly, lipomatosis, haemangiomas and genital pigmentation; and Gorlin syndrome (GS; MIM 109 400) characterised by multiple naevoid basal carcinomas, skeletal abnormalities, and odontogenic keratinocytes, macrocephaly, intracranial calcification, and craniofacial abnormalities. Compared with JPS the risk of gastrointestinal malignancy in CS, BRRS, and GS appears to be low.[4]

GS results from germline mutations in the *PTCH* gene (homologue of *Drosophila* patched) on chromosome 9q22.1.[5] Juvenile polyps appear to comprise a relatively minor and infrequent component of this disease although few GS patients undergo gastrointestinal screening. Nevertheless, *PTCH* remains a good candidate for JPS given the possibility that a different spectrum of mutations might cause juvenile polyps without the other features of GS. No study to date has tested *PTCH* for germline mutations in JPS patients.

The CS gene is *PTEN* (phosphatase and tensin homologue deleted on chromosome 10 (10q22-q23)),[6] a ubiquitously expressed dual specificity phosphatase that acts as a tumour suppressor and is mutated in several sporadic tumour types.[7–12] The inference from patients' clinical features that CS and BRRS might represent allelic forms of the same disease is supported by demonstration of germline mutations in *PTEN* in some[13–15] but not all patients with BRRS.[16] The shared clinical features of CS/BRRS and JPS, coupled with coincident somatic mutation data in juvenile polyps,[17] raised the possibility that *PTEN* could cause all of these syndromes. This hypothesis has been

Abbreviations used in this paper: JPS, juvenile polyposis syndrome; CS, Cowden syndrome; BRRS, Bannayan-Ruvalcaba-Riley syndrome; GS, Gorlin syndrome; CSGE, conformation specific gel electrophoresis; SSCP, single strand conformational gel polymorphism.

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pursued by a number of workers with differing findings.[18–22] Some studies found germline *PTEN* mutations in individuals who had been diagnosed as having JPS but there is doubt that a diagnosis of CS/BRRS had been formally excluded in these patients. We have previously found no *PTEN* mutations in a relatively large set of JPS patients (including a subset of the cases reported below).

Recently it has been shown that constitutional mutations in *DPC4* (deleted in pancreatic carcinoma locus 4; also referred to as *SMAD4*, small mothers against decapentaplegic deleted in pancreatic carcinoma, locus 4) can cause JPS.[23 24] This gene, which codes for a protein involved in transforming growth factor β signal transduction, is mutated in a number of gastrointestinal cancers.[25 26] Previous studies have reported *DPC4* mutations in 5/9 (56%) and 1/21 (5%) JPS patients (the latter study including a subset of the cases reported below). Other members of the SMAD family are candidates for JPS but no mutations in *SMAD1/2/3/5/6/7* have been found in JPS patients.[27]

Hereditary mixed polyposis syndrome (HMPS, MIM 601 228) is also characterised by atypical juvenile polyps with mixed features of hamartomas and adenomas.[28] No gene for this syndrome has been identified but linkage to chromosome 6q has been reported in one large family.[29]

Hence there is evidence of considerable genetic heterogeneity in JPS. We have studied 56 individuals with a presumptive diagnosis of JPS, comprising 47 cases from 15 JPS families and nine apparently sporadic cases. These patients comprise an exceptionally large set of JPS cases. Our aims were: (i) to describe the clinical features of JPS patients (including features overlapping with CS, BRRS, and GS); (ii) to resolve the debate as to whether or not *PTEN* mutations account for any cases of JPS; (iii) to test *PTCH* as a candidate gene for JPS; (iv) to determine the contribution of *DPC4* mutations to JPS; (v) to assess the existence of any associations between germline mutations and clinical features; and (vi) to determine the proportion of JPS cases caused by as yet unidentified genes.

Patients and methods

Patients with JPS were identified from sources in the UK, Australia, Israel, USA, Japan, and Korea. The diagnostic criteria for JPS used in this study were in accordance with the proposal of Ko and colleagues[30] that affected individuals have either five or more juvenile polyps throughout the gastrointestinal tract or any number of juvenile polyps and a family history of JPS. All affected individuals had more than one typical juvenile polyp as confirmed by histology. The following clinical data were also obtained using a standardised proforma: family history; occurrence of benign and malignant tumours; dysmorphic features; arteriovenous malformations and other cardiovascular anomalies; pigmentation; and other notable clinical features including those of CS, BRRS, and GS. Where possible, material from JPS polyps and other tumours was obtained for histological review and confirmation of diagnosis.

Constitutive DNA from individuals was extracted from EDTA blood samples using a standard sucrose lysis method. Published oligonucleotide and reaction conditions were used to amplify specifically each exon of the *DPC4*, *PTEN*, and *PTCH* genes.[20 24 31] The search for germline mutations in *DPC4* was performed using conformation specific gel electrophoresis (CSGE). Two independent workers using either CSGE or single strand conformational gel polymorphism (SSCP) undertook screening for mutations in *PTEN*. A combination of CSGE and SSCP was used to screen the *PTCH* gene. All samples with band shifts were sequenced in duplicate and in forward and reverse orientations, after reamplification of the appropriate exon from genomic DNA in the polymerase chain reaction, using the ABI Ready Reaction Dye Terminator Cycle Sequencing kit and the 377 Prism sequencer.

Results

Fifty six patients with JPS were studied; 47 were ascertained from 15 families and nine apparently sporadic patients had no known relatives affected with JPS, although in three cases there was a strong family history of colorectal cancer. Table 1 details the clinical characteristics of all patients. Mean age at presentation of juvenile polyposis was 23 (SD 16) years (range 1–65). All affected individuals from whom histological material was available had juvenile polyps of a typical type.

The extent of polyposis within the gastrointestinal tract varied considerably between families in terms of both the numbers and sites of polyp formation. Some of the families showed marked evidence of intrafamilial differences in disease expression (table 1) even allowing for factors such as patient age and screening by colonoscopy. Furthermore, in a number of patients there was documented evidence of adenomatous intestinal polyposis although none had the atypical juvenile polyps found in HMPS.

The increased risk of gastrointestinal cancer reported in association with JPS was clearly present in our patients. Fifteen (27%) had developed some type of gastrointestinal cancer by the age of 65. Of these, six were small bowel carcinomas, two gastric cancers, and seven colorectal cancers. Based on cancer incidence rates in the UK,[32] this equates to about a 16-fold increased risk of gastrointestinal malignancy in JPS.

None of the patients had prototypical dermatological (e.g. tricholemmomas) or skeletal phenotypic features indicative of CS, BRRS, or GS. There was some overlap between the clinical features of JPS patients and these other syndromes—three patients had macrocephaly for example—but no specific features to suggest misdiagnosis. Furthermore, none of the patients had a past history of thyroid cancer and only one had developed breast cancer. Three patients had hyper- or

hypothyroidism but no tissue diagnosis suggestive of CS. The increased risk of cancer in these JPS patients appeared to be confined to the gastrointestinal tract in contradistinction to CS and GS. Arteriovenous and other cardiovascular anomalies were present in three patients (5%). The reported association between JPS and these anomalies may therefore be genuine.

Table 1 Clinical features of the patients studied

				Phenotype								
Family	*ID*	*Age**	*Sex*	*Gastrointestinal tract***	*Skin*	*Skeletal*	*CNS*	*Cardiac*	*Breast*	*Thyroid*	*Other*	*Additional information*
W	3	47	F	Caecal Ca aged 47, jejunal JPs and adenomas, TA								
W	2	14	F	100+JPs								
W	1	32	F	Ca colon (transverse) aged 32,					BBD	Hyper		
SK	1	13	F	Multiple colonic polyps (mixed, Ad and JPs)								
SRO	1	13	F	Multiple JPs							P	
SS	1	29	F	(50+) JPs								Niece Ca colon (25); sister Ca pancreas; father Ca larynx and stomach
SV	1	12	M	Sigmoid and rectal polyps (50+)								Father Ca colon aged 48; Pat. uncle Ca colon aged 50
SH	1	1	M	Multiple colonic polyps, resection aged 8		CLP, PD						
SC	1	16	M	Colonic (30-50)		MC						
SD	1	15	M	Jejunal polpys				CD				
SR	1	4	F	Recto-sigmoid and Caecal polyps (50+)				VSD				
AF	1	16	M	Colonic polyps (4)								Father JPs and Ca colon***
SCA	1	27	F	Multiple sigmoid polyps								
3	3.5	37	M	Colonic and gastric polyps								
	4.2	6	M	Ileal, jejunal, colonic, and rectal polyps; Ca ileum; Ca stomach								
	2.3	65	F	Colonic and rectal polyps	ML							
10	102	41	F	Ca colon								
	202	21	M	Ileum and colon								
	201	24	M	Multiple								
1	204	32	F	Small bowel, colonic and gastric polyps (JPs and Ad, 20+); Ca ileum aged 49; intussusception								Sister, lymphoma aged 42, Ca breast aged 54.
	302	16	M	Colonic polyps								
	301	21	F	Colonic polyps (20)								
6	302	23	M	Colonic and small intestinal polyps (50+)		MC						
	402	9	F	Colonic polyps								
	201	55	F	Colonic polyps (3)								
	401	12	M	Colonic polyps (2)								
11	4.4	18	M	Colonic polyps (3)							HT	
	3.5	30	M	Colonic polyps	FK						SR	
8	1.2	50	F	Ca colon aged 50								
	3.1	20	M	Caecal polyps								
	2.1	18	F	Multiple JPS; Ca stomach								
9	301	17	M	Colonic polyps								
	203	19	F	JPs and Ad polyps in colon							BK	
	302	4	M	Colonic polyps				AS and CO				
15	502	14	M	Multiple colonic polyps		CL						
	305	51	F	Ca colon (transverse) aged 51, multiple colonic polyps						Hypo		Brother Ca colon aged 38, multiple colonic polyps
	306	43	M	Colonic polyps (10)								
	308	61	M	Caecal Ca aged 61								
	405	48	F	Multiple colonic polyps		CL		AR		Hyper	OVC	
12	204	8	M	Multiple colonic polyps; Ca jejunum			SAH					
	206	8	M	Multiple colonic polyps								
	201	7	M	Polyposis at autopsy								
	307	4	F	Florid colonic polyposis; small intestinal polyps (22); Ca small intestine aged 27.							OVC	
	304	22	F	Caecal and colonic polyps								
	308	17	M	Multiple (TA, Ad and JP) in colon and duodenum; Ca jejunum		MC						
	203	7	M	Ca jejunum aged 37			Schiz					
16	102	19	F	Ca colon aged 60				BBD				
	301	13	F	20+ polyps								
	302	14	M	Multiple colonic polyps								
	201	32	M	Multiple polyps in colon and stomach								
17	202	NK	M	>100 gastric JPs, 8 colorectal JPs								
	203	NK	M	Gastric JPs	ML							
20	201	NK	F	Extensive polyposis, colectomy aged 45								
	301	NK	F	Colorectal JPs; colectomy aged 21							OVC	
BL	101	39	M	Colonic polyps (TA +Ad)								
	201	6	F	>100 JP, exocrine pancreatic insufficiency								Short stature, development delay

*At diagnosis (NK, not known); **number of polyps in parentheses; ***familial case but only index case analysed.
Ad, adenoma; AR, aortic regurgitation; AS, aortic stenosis; BBD, benign breast disease; BK, bifid kidney; Ca, Cancer; CD, conduction defect; CLP, cleft palate; CL, clinodactylyl; CO, coarctation; FK, excessive freckling; HT, hypertelorism; Hyper, hyperthyroidism; Hypo, hypothyroidism; JPs, juvenile polyps; MC, marcocephaly; ML, multiple lipomas; OVC, ovarian cyst; P, poryphria; PD, polydactylyl; SAH, subarchnoid haemorrhage; Schiz, schizophrenia; SR, Schatski ring; TA, tubular adenoma; VSD, ventricular septal defect.

Table 2 Description of DPC4 mutations identified in JPS patients

Patient/ family	*Mutation*	*Predicted effect*
SV	11bp deletion (GTCCACTGAAG) at nucleotide 516 (exon 4)	Frameshift creating stop codon at nucleotide 561
AF	CGC to TGC, at nucleotide 1083 (exon 8)	Arg to Cys
17	2 bp deletion (CC) at nucleotide 1564 (exon 11)	Frameshift creating stop codon at nucleotide 1575
20	9 bp deletion (AAATGGAGC) at nucleotide 189 (exon 1)	Deletion of amino acids 64-66
BL	CGA to TGA at nucleotide 1333 (exon 10)	Substitution creating stop codon

No JPS patient had a germline *PTEN* mutation. Control samples with known *PTEN* mutations showed aberrant SSCP bands and previously reported polymorphisms were detected. *PTEN* mutations are typically found in 80% of CS and 50% of BRRS cases which adhere to operational diagnostic criteria established by the International Cowden Consortium.[33] Also, germline *PTCH* mutations were not detected in our JPS cases. *PTCH* mutations are typically detectable in 39% of GS patients.[31]

Five germline *DPC4* mutations were identified (table 2). Three of these were deletions ranging in size from two to 11 base pairs in exons 1, 4, and 11. One of the mutations was a single base substitution creating a stop codon in exon 10. The fifth mutation was a missense mutation in exon 8. This variant has been reported previously.[24] Our series therefore confirms that mutations in *DPC4* are associated with JPS but the mutations we detected did not involve the trimerisation domain of the *DPC4* protein. There were no distinguishing clinicopathological features in those patients harbouring *DPC4* mutations.

Discussion

Several syndromes—JPS, CS, BRRS, GS, and HMPS—include juvenile polyps in their phenotype. These diseases can have subtle manifestations and some of their clinical features are not unique. Hence diagnosis is not straightforward. However, it is important to distinguish between these syndromes as the types of cancer associated with each appear to be quite different. A more reliable and objective means of differentiating syndromes than reliance solely on clinical features is clearly desirable, especially in the case of JPS the diagnosis of which is made in part by exclusion. Determining the molecular basis of each of these syndromes clearly offers the best method of establishing a diagnosis.

Until recently the molecular basis of the hamartomatosis polyposis syndromes was unknown. Early somatic data suggested the existence of a putative locus for JPS at 10q22–24, encompassing *PTEN*.[17] This observation led several researchers to examine *PTEN* as a candidate for JPS. No linkage to 10q22 or mutation in *PTEN* was detected in either the JPS families or isolated cases studied by Marsh and colleagues.[20] However, both Olschwang and colleagues[21] and Lynch and colleagues[18] reported germline *PTEN* mutations in patients with multiple juvenile polyps, suggesting that *PTEN* was the cause of some JPS cases. Eng has recently questioned their conclusion.[33] In the study by Olschwang and colleagues,[21] the adult male patient (G116) also had a laryngeal carcinoma and a heterogeneous thyroid nodule, which is highly suggestive of CS. Furthermore, although both children in the report (G796 and G710) did not have features indicative of CS or BRRS, they were less than 15 years of age. As the penetrance of CS is only 10% by this age[34] it is possible that both children will develop features of CS later in life. Similarly, the affected members of the family reported by Lynch and colleagues[18] had major clinical features consistent with a diagnosis of CS.

Our mutational analysis of JPS patients is the most comprehensive to date. The findings concur with previous work suggesting that the probability of detecting a constitutive mutation in *PTEN* is not high if a patient's clinical phenotype does not adhere to the International Cowden Consortium operational diagnostic criteria for CS[33] or to the specific clinical features of BRRS.

PTCH is a good candidate for JPS but has not previously been tested for germline mutations in JPS patients. We have excluded this gene as a cause of juvenile polyps outside the setting of GS. Although *PTCH* mutations may directly lead to gastrointestinal hamartoma formation it remains possible that reports of juvenile polyps in GS result from a chance association or from contiguous deletion of *PTCH* and at least one other gene.

Recent work has identified germline mutations in the *DPC4* (*SMAD4*) gene on 18q21.1 as a cause of some cases of JPS.[23] In our study 21% of JPS could be ascribed to germline mutations in *DPC4* (five of 24; comprising 15 families and nine sporadic cases tested). This provides further support for the role of mutations in this gene as a cause of JPS. Failure to detect mutations in *DPC4* in the other patients is unlikely to reflect problems in the screening method alone: it is possible that some mutations may be in the UTRs, introns, or promoter region of the gene but we have screened all exons and splice sites and have found that CSGE can detect all small insertions, deletions, and 90% of single base substitutions under such conditions.

DPC4/*SMAD4* is a pivitol component of the transforming growth factor β signal transduction pathway.[35] Through hetero-oligomer formation by interaction with *SMAD1* (and possibly 5) and *SMAD2* (and possibly 3), *SMAD4* mediates apoptotic and growth inhibition responses. Hamartoma formation in JPS presumably results from disruption of the transforming growth factor β signal transduction pathway.[35] In our study mutations in *SMAD4* were detected in exons 1, 4, 8, 10, and 11. Pathogenic mutations previously reported were in exons 5, 8, and 9.[23] A wide range of mutations can clearly affect cellular responses to transforming growth factor β leading to hamartoma formation. Whether specific genotype-phenotype relationships exist await further studies.

We conclude that the clinical features of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Gorlin syndrome can be used to distinguish patients who are likely to carry germline mutations in *PTEN* and *PTCH* and who do not, therefore, have a diagnosis of JPS. The rare syndrome HMPS can probably be distinguished from JPS on histological grounds, and there is no evidence for linkage of JPS to the HMPS locus (although this cannot be confirmed until the HMPS gene is identified). Combining our findings and those reported by Howe and colleagues[23] suggests that up to 40% of JPS cases might be caused by germline mutations in *DPC4* although these patients cannot be distinguished from the majority of JPS patients who harbour germline mutations in uncharacterised genes. Current evidence does not suggest any common mode of action of *PTEN*, *PTCH*, and *DPC4*. Therefore, there are few clues as to the nature of the uncharacterised genes for JPS as it appears that several different cellular defects are associated with hamartomatous juvenile polyps of identical appearance.

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