Genetics inMedicine | ARTICLE

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Disease expression in juvenile polyposis syndrome: a retrospective survey on a cohort of 221 European patients and comparison with a literature-derived cohort of 473 *SMAD4/BMPR1A* pathogenic variant carriers

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Purpose: Juvenile polyposis syndrome (JPS) is a rare, autosomal-dominantly inherited cancer predisposition caused in approximately 50% of cases by pathogenic germline variants in *SMAD4* and *BMPR1A*. We aimed to gather detailed clinical and molecular genetic information on JPS disease expression to provide a basis for management guidelines and establish open access variant databases.

Methods: We performed a retrospective, questionnaire-based European multicenter survey on and established a cohort of *SMAD4/BMPR1A* pathogenic variant carriers from the medical literature.

Results: We analyzed questionnaire-based data on 221 JPS patients (126 kindreds) from ten European centers and retrieved literature-based information on 473 patients. Compared with *BMPR1A* carriers, *SMAD4* carriers displayed anemia twice as often (58% vs. 26%), and exclusively showed overlap symptoms with hemorrhagic telangiectasia (32%) and an increased prevalence (39% vs. 13%) of gastric juvenile polyps. Cancer, reported in 15% of JPS patients (median age 41 years), mainly occurred in the colorectum (overall: 62%, *SMAD4*: 58%, *BMPR1A*: 88%) and the stomach (overall: 21%; *SMAD4*: 27%, *BMPR1A*: 0%).

Conclusion: This comprehensive retrospective study on genotype–phenotype correlations in 694 JPS patients corroborates previous observations on JPS in general and *SMAD4* carriers in particular, facilitates recommendations for clinical management, and provides the basis for open access variant *SMAD4* and *BMPR1A* databases.

Genetics in Medicine (2020) 22:1524–1532; https://doi.org/10.1038/s41436-020-0826-1

Keywords: polyposis; juvenile polyposis syndrome; colorectal cancer; genotype–phenotype correlation; hereditary hemorrhagic telangiectasia

INTRODUCTION

With an estimated incidence of 1:16,000 to 1:100,000, juvenile polyposis syndrome (JPS; OMIM 174900) represents a rare, autosomal-dominantly inherited cancer predisposition syndrome in which patients develop numerous juvenile type hamartomatous polyps (JPs) in the gastrointestinal (GI) tract and are at increased lifetime risk of 9–50% for GI cancers, mostly of the colon.[1–3] The clinical diagnosis is established if an individual develops synchronously and/or metachronously five or more colorectal JPs, multiple JPs throughout the GI tract, or any number of JPs and a family history of juvenile polyposis.[4] JPS is particularly prone to misdiagnosis (e.g., small juvenile polyps are often misdiagnosed as inflammatory polyps because they lack the diagnostic features of juvenile type polyps) and differentiation from PTEN hamartoma tumor syndrome (PHTS) can be challenging. Therefore,

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Submitted 27 September 2019; revised 27 April 2020; accepted: 27 April 2020
Published online: 13 May 2020

multigene panel diagnostics in patients with clinical suspicion of PHTS should also include *SMAD4* and *BMPR1A*.

In 45–55% of all JPS patients the clinical diagnosis can be confirmed by identification of a germline pathogenic variant (PV) in either the *SMAD4* (Locus Reference Genomic [LRG] ID LRG_318t1) or the *BMPR1A* (LRG ID LRG_298t1) gene, with the majority consisting of missense and nonsense variants, small insertions/deletions, and large genomic deletions.[5–7] Both proteins belong to the transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) superfamily of molecules with SMAD4 serving as central mediator of TGF-β and BMP signaling.[8] While *SMAD4* loss-of-function alterations have been associated with tumorigenesis as well as with a juvenile polyposis–hereditary hemorrhagic telangiectasia overlap syndrome (JPS-HHT; OMIM 175050[9]), *SMAD4* de novo gain-of-function variants result in autosomal-dominant Myhre syndrome, a connective tissue disorder with multisystem involvement and intellectual disability (OMIM 139210[10]). Moreover, microdeletions encompassing both the *BMPR1A* and the *PTEN* gene loci on chromosome 10q22-q23 have been described in patients presenting with early-onset JPS and features of PTEN hamartoma tumor syndrome, the severity depending on the extent of the deletion.[11] The pathogenetic events behind *SMAD4*- and *BMPR1A*-associated juvenile polyposis are still poorly understood; only scarce data are available on the putative roles of these genes as tumor suppressors and the nature of the respective somatic alterations (2nd hit) involved in polyp formation.[12–15]

Despite being known for more than 50 years[16] clinical guidelines for JPS, due to its rarity, still essentially depend on clinical and genetic information gathered from single series/center reports, mostly focused on *SMAD4* PV carriers, and expert opinion.[17] Additionally, there exists no comprehensive, open access database on genetic variants in *SMAD4* or *BMPR1A*. The aims of this retrospective study were to describe the clinical and molecular genetic features from a European, multicenter survey on JPS patients, compare them with literature-derived data on *SMAD4*/*BMPR1A* PV carriers, and provide recommendations for management on the basis of both new and previously published data. In addition, the data establish the basis for *SMAD4* and *BMPR1A* Leiden Open Variant Databases (LOVD).

MATERIALS AND METHODS

European collaborative study (ECS) group

Enrollment and data collection

Ten medical centers from eight European countries participated in this retrospective, European collaborative study (ECS) on JPS and contributed patient data in an anonymized fashion based on a questionnaire that specifically asked for clinical, familial, and genetic details (Supplementary Table 1 and Supplementary Fig. A). The study was conducted with the approval of the Ethikkommission beider Basel ("Basler Studie ueber familiaere Tumorkrankheiten," number 258/05) and all collaborating centers had obtained local approval for the study according to national guidelines, adhering to the principles set out in the Declaration of Helsinki. Informed consent was obtained from all patients. Only patients who fulfilled the clinical criteria for JPS and in whom a disease causing, i.e., likely pathogenic (class 4) or pathogenic (class 5) *SMAD4* or *BMPR1A* germline variant could be identified were submitted. Thirty JPS patients had to be excluded from the study because of incomplete genetic and/or clinical data. The final ECS group comprised 221 patients from 126 kindreds (1 to 9 patients per kindred).

Literature-based *SMAD4/BMPR1A* PV carrier (LBSB) group

Search and selection criteria

To retrieve all published *SMAD4/BMPR1A* carriers of PVs reported in the medical literature, a comprehensive search for entries between 1 January 1998 and 31 December 2018 was performed using six databases: CINAHL, EMBASE, MEDLINE/PubMed, PsycINFO, Psyndex, and Web of Science. Each database was searched individually for genotype and phenotype characteristics using both free text searches as well as entry term searches (Supplementary Fig. B). Following the selection process depicted in Supplementary Fig. C and exclusion of (1) patients with promoter or unspecified *SMAD4/BMPR1A* variants, (2) patients with *SMAD4*-associated Myhre syndrome ($n = 8$), and (3) ECS group patients having already been published ($n = 39$), 628 *SMAD4/BMPR1A* variant carriers could be retrieved. Applying variant classification criteria based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines[18] a total of 473 patients (249 *SMAD4*, 224 *BMPR1A*) carrying pathogenic (i.e., class 4 and 5) gene variants were included, and phenotype data, as for the ECS group, were gathered, where available, from the pertinent publications for comparison.

Categorization of polyp numbers reported

Information on polyp numbers was summarized in three categories: "few," "multiple," and "massive." "Few" encompasses descriptions such as "few," "some," or 1–4 polyps; "multiple" such as "multiple," "numerous," "several," "many," or 5–99 polyps; and "massive" containing "massive," "diffuse," "carpeted," "lots," or ≥100 polyps.

Statistical methods

Statistical comparison of patients' features, encompassing phenotypic characteristics and molecular status, was done using Chi-square and Fisher's exact tests for categorical variables (e.g., gender, presence of symptoms), or Student's *t* test for continuous variables (e.g., age at diagnosis), with all of the probabilities reported as two-tailed *P* values, considering $p < 0.05$ to be statistically significant. All calculations were done either in Microsoft Excel 2016 or Stat View v.4.5 (Abacus Concepts).

RESULTS

The 221 ECS patients (sex ratio: m:f = 105:116) from 126 kindreds consisted of 127 patients with a germline PV in the

SMAD4 gene (57.5%; 71 kindreds; 56 nonindex) and 94 patients with a PV in the *BMPR1A* gene (42.5%; 55 kindreds; 39 nonindex). Having excluded previously published ECS patients, the LBSB group consisted of 473 patients (224 *BMPR1A*; 249 *SMAD4*) with individual phenotypic information ascertained from 232 (49.1%) PV carriers (sex ratio [$n = 160$]: m:f = 80:80).

Age at diagnosis

The median age at JPS diagnosis of all ECS patients was 25.0 years (interquartile range [IQR] 28.0, range 0.2–86 years) with a similar age at diagnosis for *SMAD4* (28.0 years) and *BMPR1A* (24.5 years) PV carriers (Supplementary Table 2). A significant difference in age at diagnosis was seen between index patients (19.5 years; IQR 23.5, range 0.6–86 years) compared with other family members, referred to as nonindex patients (32.0 years; IQR 30.5, range 0.2–76 years; $p = 0.0017$).

While median age at JPS diagnosis was similar for LBSB patients in general (23.0 years, IQR 27.0; $n = 104$) and for *SMAD4* carriers (26.0 years, IQR 26.5; $n = 71$), carriers of large cytogenetic alterations, i.e., microdeletions at 10q23 encompassing the *BMPR1A* and *PTEN* gene loci, displayed a significantly younger age (1.5 years, IQR 2.6; $n = 8$) compared with carriers of other *BMPR1A* pathogenic variants (23.0 years, IQR 26.0; $n = 26$; $p < 0.001$). This was also observed in the five ECS index patients with 10q23 microdeletions whose median age at diagnosis was 6.0 years (IQR 5.0) compared with carriers of other types of *BMPR1A* variants (21.0 years; IQR 19.0; $n = 48$; $p = 0.009$).

Symptoms at presentation

The most common symptoms reported in ECS index patients were rectal bleeding (43%) and anemia (47%). Whereas the former was nearly equally frequent in *SMAD4* (41%) and *BMPR1A* (46%) PV carriers, anemia was reported twice as often in *SMAD4* carriers (58% vs. 26%, $p = 0.0003$; Table 1), who also exhibited symptoms of hereditary hemorrhagic telangiectasia in 32% (13/41). In ECS nonindex patients, these symptoms were significantly less common in *SMAD4* (14%; 11%; $p = 0.006$) and absent in *BMPR1A* PV carriers (0% for both; $p < 0.0001$). Symptoms associated with JPS-HHT, such as epistaxis, telangiectasia, or other types of arteriovenous malformations, were exclusively reported in *SMAD4* PV carriers (24%, 21%, and 14% of index patients, respectively) but not in *BMPR1A* PV carriers (0%). A similar pattern was observed in LBSB patients (*SMAD4* [$n = 55$]: 16.1%, 10.8%, and 12.6%; *BMPR1A*: 0%).

Congenital heart defects (CHD), mainly ventricular and atrial septal defects, were reported in 3 (4.2%) *SMAD4* and 5 (9.1%) *BMPR1A* index patients ($p = 0.29$; Table 1). Overall, CHD was more frequently stated in *BMPR1A* compared with

Table 1 Symptoms at presentation.

		Index patients			Nonindex patients		
		Total[a]	Total with feature[b]		Total[a]	Total with feature[b]	
		n	*n* (% of total)	Median age (years)[c] (IQR)	*n*	*n* (% of total)	Median age (years)[c] (IQR)
Intestinal	Blood in stool	126	54 (42.9%)	11.5 (15.0)	95	8 (8.4%)	17.0 (21.5)
	SMAD4	71	29 (40.8%)	11.0 (13.0)	56	8 (14.3%)	17.0 (21.5)
	BMPR1A	55	25 (45.5%)	12.0 (17.3)	39	0	-
	Anemia	126	55 (46.7%)	17.5 (26.0)	95	6 (6.3%)	35.5 (11.0)
	SMAD4	71	41 (57.7%)	18.0 (27.5)	56	6 (10.7%)	35.5 (11.0)
	BMPR1A	55	14 (25.5%)	15.0 (30.8)	39	0	-
Extraintestinal, vascular	Epistaxis	126	17 (13.5%)	11.0 (31.0)	95	8 (8.4%)	23.0 (33.8)
	SMAD4	71	17 (23.9%)	11.0 (31.0)	56	8 (14.3%)	23.0 (33.8)
	BMPR1A	55	0		39	0	-
	Telangiectasia	126	15 (11.9%)	38.0 (18.5)	95	2 (2.1%)	32.5 (33.0)
	SMAD4	71	15 (21.1%)	38.0 (18.5)	56	2 (3.6%)	32.5 (33.0)
	BMPR1A	55	0		39	0	-
	AVM	126	10 (7.9%)	46.5 (12.0)	95	3 (3.2%)	31.0 (14.0)
	SMAD4	71	10 (14.1%)	46.5 (12.0)	56	3 (5.4%)	31.0 (14.0)
	BMPR1A	55	0		39	0	-
Cardiac	Congenital heart defects	126	8 (6.3%)	6.0 (12.0)	95	3 (3.2%)	1.0 (0.0)
	SMAD4	71	3 (4.2%)	23 (0.0)	56	0	-
	BMPR1A	55	5 (9.1%)	4.5 (14.0)	39	3 (7.7%)	1.0 (0.0)

AVM arteriovenous malformation, *IQR* interquartile range.
[a]Total number of patients.
[b]Total number of index or nonindex patients who presented with the respective symptom.
[c]Due to missing age at diagnosis the calculation of the median age is often based on fewer than the total number of patients with the respective feature.

Table 2 Polyp occurrence in the GI tract.

	Index patients			Nonindex patients		
	Total[a]	Total with feature[b]		Total[a]	Total with feature[b]	
	n	*n* (% of total)	Median age (years)[c] (IQR)	*n*	*n* (% of total)	Median age (years)[c] (IQR)
JP in stomach	126	35 (27.8%)	40.0 (16.0)	95	23 (24.2%)	42.0 (19.0)
SMAD4	71	28 (39.4%)	40.0 (13.5)	56	21 (37.5%)	41.0 (14.8)
BMPR1A	55	7 (12.7%)	35.5 (28.0)	39	2 (5.1%)	56.5 (11.0)
JP in small intestine	126	13 (10.3%)	24.0 (26.5)	95	10 (10.5%)	46.0 (22.5)
SMAD4	71	10 (14.1%)	29.0 (18.5)	56	10 (17.9%)	46.0 (22.5)
BMPR1A	55	3 (5.5%)	14.0 (30.0)	39	0	
JP in colon	126	111 (88.1%)	18.0 (25.0)	95	45 (47.4%)	20.0 (30.8)
SMAD4	71	61 (85.9%)	19.0 (24.5)	56	29 (51.8%)	30.5 (35.0)
BMPR1A	55	50 (90.9%)	18.0 (23.3)	39	16 (41%)	16.0 (31.0)
JP in rectum	126	58 (46.0%)	18.5 (28.0)	95	12 (12.6%)	13.0 (15.0)
SMAD4	71	30 (42.3%)	14.0 (28.8)	56	7 (12.5%)	6.0 (11.8)
BMPR1A	55	28 (50.9%)	19.0 (27.8)	39	5 (12.8%)	15.0 (22.0)
Other types of polyps[d]	126	58 (46.0%)	23.0 (23.0)	95	25 (26.3%)	40.0 (29.0)
SMAD4	71	30 (42.3%)	23.0 (24.0)	56	16 (28.6%)	42.0 (25.0)
BMPR1A	55	28 (50.9%)	23.0 (20.5)	39	9 (23.1%)	31.0 (41.0)

GI gastrointestinal, *IQR* interquartile range, *JP* juvenile polyp.
[a]Total number of patients.
[b]Total number of index or nonindex patients who presented with the respective symptom.
[c]Due to missing age at diagnosis the calculation of the median age is often based on fewer than the total number of patients with the respective feature.
[d]Adenomas, serrated and other polyps.

SMAD4 PV carriers (8.5% [8/94] vs. 2.4% [3/127]; $p = 0.06$), which was also the case in the LBSB group (4.0% [9/224] vs. 0.4% [1/249]; $p = 0.008$). The small numbers precluded any meaningful statistical/genotype–phenotype analysis; we did not, however, observe an overrepresentation of a specific type of PV or protein domain affected. Aortic aneurysms were exclusively reported in *SMAD4* PV carriers (ECS group: 2 [1.6%], LBSB group: 1 [0.4%]).

Few ECS and LBSB patients displayed other symptoms such as macrocephaly ($n = 11$, with 9 of them carrying a 10q32 microdeletion encompassing the *BMPR1A* and *PTEN* gene loci), failure to thrive ($n = 9$), and protein-losing enteropathy (5). Except for a LBSB group patient with a 10q32 microdeletion, no further cases of cleft palate or polydactyly were reported.

Polyp occurrence in the GI tract

Juvenile polyps in the colorectum

A high prevalence of juvenile polyps (JPs) in the colon was reported in ECS index patients (*BMPR1A*: 91% and *SMAD4*: 86%), essentially mirrored by the LBSB group (96% [42/44] and 93% [69/74]), whereas this was significantly less frequently the case for ECS nonindex patients (41% and 52%, respectively; $p < 0.0001$; Table 2). JPs were more frequently reported in the proximal than the distal colon, both in index (40/70; 57.1%) and clinically affected nonindex (13/19; 68.4%) patients (Table 3). The load of colonic JPs was similar in both groups, ECS and LBSB, as well as in the gene-specific subgroups, with most index patients presenting with multiple (5 to 99) JPs (Table 3). In the ECS group, median age at diagnosis was similar for index and nonindex patients (18.0 years and 20.0 years). Interestingly, *BMPR1A* index and nonindex patients displayed a similar median age at diagnosis, compared with *SMAD4* carriers where nonindex patients were diagnosed about 11.5 years later. Unfortunately, for most LBSB patients specific information regarding age at diagnosis and polyp occurrence could not be retrieved from the respective publications, which precluded meaningful calculations in the corresponding LBSB subgroups.

Compared with those in the colon, rectal JPs in the ECS group overall were significantly less frequently reported (70.6% [$n = 156$] vs. 31.7% [$n = 70$]; $p < 0.01$) and appeared to be somewhat more prevalent in *BMPR1A* index patients (Table 2). In nonindex patients median age at diagnosis of rectal JP was lower (13.0 years) compared with colonic JP (20.0 years, $p = 0.15$; Table 4). Rectal polyp load was similar among *BMPR1A* and *SMAD4* PV carriers, with the majority of patients presenting with few (<5) to multiple (5 to 100) JPs (Table 3).

Juvenile polyps in the stomach and in the small bowel

As specific information regarding the presence of JP in the upper GI tract is largely missing in the LBSB patients, we only report the findings in the ECS patients. A clear genotype–phenotype correlation was observed for gastric juvenile polyposis, which was significantly more frequent in *SMAD4* than *BMPR1A* PV carriers, both in index and nonindex patients (*SMAD4* 39% and 38% vs. *BMPR1A* 13% and 5%; $p = 0.001$ and 0.0002, respectively; Table 2). JP formation in the stomach was also more severe in *SMAD4*

Table 3 Number of juvenile polyps according to GI tract site.

	Index patients			Nonindex patients		
	Total	*SMAD4*	*BMPR1A*	Total	*SMAD4*	*BMPR1A*
Stomach	**31**	24	7	**17**	15	2
Few	9	3 (13%)	6 (86%)	7	5 (33%)	2 (100%)
Multiple	18	17 (71%)	1 (14%)	6	6 (40%)	0
Massive	4	4 (17%)	0	4	4 (27%)	0
Small intestine	**10**	7	3	**9**	9	0
Few	6	5 (71%)	1 (33%)	5	5 (56%)	0 (%)
Multiple	4	2 (29%)	2 (67%)	4	4 (44%)	0 (%)
Massive	0	0	0	0	0	0
Colon	**92**	49	43	**37**	23	14
Few	11	7 (14%)	4 (9%)	9	5 (22%)	4 (29%)
Multiple	69	33 (68%)	36 (84%)	27	17 (74%)	10 (71%)
Massive	12	9 (18%)	3 (7%)	1	1 (4%)	0
Proximal colon	**40**	25	15	**13**	8	5
Few	7	5 (20%)	2 (13%)	7	5 (63%)	2 (40%)
Multiple	32	19 (76%)	13 (87%)	6	3 (37%)	3 (60%)
Massive	1	1 (4%)	0	0	0	0
Distal colon	**30**	20	10	**6**	3	3
Few	14	9 (45%)	5 (45%)	2	0	2 (67%)
Multiple	15	10 (50%)	5 (55%)	4	3 (100%)	1 (33%)
Massive	1	1 (5%)	0	0	0	0
Rectum	**38**	20	18	**8**	3	5
Few	16	11 (50%)	5 (28%)	6	3 (100%)	3 (60%)
Multiple	22	9 (50%)	13 (72%)	2	0	2 (40%)
Massive	0	0	0	0	0	0
Other polyps	**48**	25	23	**16**	12	4
Few	15	5 (20%)	10 (43%)	8	5 (42%)	3 (75%)
Multiple	29	18 (72%)	11 (48%)	8	7 (58%)	1 (25%)
Massive	4	2 (8%)	2 (9%)	0	0	0

GI gastrointestinal.

pathogenic variant carriers since most *BMPR1A* PV carriers (86%) presented with only few (<5) polyps, whereas most *SMAD4* PV carriers (71%) presented with multiple JPs or even a massive (≥100) number of JPs (17%; $p = 0.0001$; Table 3). Combining the data from index and nonindex patients, who displayed similar JP loads, the difference remained statistically significant ($p = 0.0002$). JP formation in the small intestine was also more often reported in *SMAD4* (15.7%) than *BMPR1A* PV carriers (3.2%; Table 2).

Age at diagnosis of JP in the GI tract

The median age at JP diagnosis in the different sections of the GI tract did not significantly differ between ECS-*SMAD4* and ECS-*BMPR1A* PV carriers. A significant difference was only observed for index patients with stomach polyps where the reported median age at diagnosis was roughly 20 years later (40.0 years) compared with colonic (18.0 years; $p < 0.0001$; Table 2).

Other polyps

In 37.6% of ECS and 82.2% of LBSB patients other types of polyps were reported, mostly adenomas and hyperplastic polyps, with similar frequencies in *SMAD4* and *BMPR1A* PV carriers (ECS: 36.2% and 39.4%; LBSB: 72.7% and 96.6%, respectively; Table 2).

Cancer occurrence

Cancer was reported in 15.4% (34/221) of ECS patients (index patients: 15.1% [19/126]; nonindex patients: 15.8% [15/95], and 11.0% [52/473] of LBSB patients; Table 4). In most ECS patients (78.1%, 25/32) the cancer diagnosis was either accompanied or preceded by the diagnosis of colonic JPs. A notable exception was found in three ECS-*SMAD4* pathogenic variant carriers, two index and one nonindex, who presented with cancer and JPs in the stomach/small intestine, but none in the colorectum; instead of JPs, two of them were reported to have multiple colorectal adenomas or hyperplastic polyps. The overall median age at cancer diagnosis was 41 years, which is 23 years later compared with the diagnosis of colon JPs (18.5 years, $p < 0.0001$) and similar to that of gastric JP (40 years). Overall, cancer was significantly more frequently observed in ECS-*SMAD4* (20.5%, $n = 26$) compared with ECS-*BMPR1A* PV carriers

Table 4 Cancer occurrence in JPS patients.

	Index patients			Nonindex patients		
	Total[a]	Total with feature[b]		Total[a]	Total with feature[b]	
	n	*n* (% of total)	Median age (years)[c] (IQR)	*n*	*n* (% of total)	Median age (years)[c] (IQR)
Cancer	126	19 (15.1%)	37.5 (14.0)	95	15 (15.8%)	45.0 (14.0)
SMAD4	71	13 (18.3%)	42.0 (16.3)	56	13 (23.2%)	43.0 (13.0)
BMPR1A	55	6 (10.9%)	34.0 (6.3)	39	2 (5.1%)	55.0 (10.0)
Colorectal cancer	126	11 (8.7%)	36.0 (7.5)	95	10 (11.6%)	49.0 (15.5)
SMAD4	71	6 (8.5%)	39.0 (13.0)	56	9 (16.1%)	46.5 (14.0)
BMPR1A	55	5 (9.1%)	35.0 (9.0)	39	2 (5.1%)	55.0 (10)
Gastric cancer	126	4 (3.2%)	44.0 (12.5)	95	3 (3.2%)	42.0 (12.8)
SMAD4	71	4 (5.6%)	44.0 (12.5)	56	3 (5.4%)	42.0 (12.8)
BMPR1A	55	0		39	0	
Multiple cancers	126	4 (3.2%)		95	2 (2.1%)	
SMAD4	71	3 (4.2%)		56	2 (3.6%)	
BMPR1A	55	1 (1.8%)		39	0	

IQR interquartile range, *JPS* juvenile polyposis syndrome.
[a]Total number of patients.
[b]Total number of index or nonindex patients who presented with the respective symptom.
[c]Due to missing age at diagnosis the calculation of the median age is often based on fewer than the total number of patients with the respective feature.

(8.5%; $n = 8$; $p = 0.015$), which could be attributed to the difference in cancer occurrence among nonindex patients (23% vs. 5%, respectively; $p = 0.022$).

Colorectal cancer ($n = 21$; median age at diagnosis: 38 years; range 19–67 years) was reported in 11.8% (15/127) of ECS-*SMAD4* and 7.4% (7/94) of ECS-*BMPR1A* carriers (Table 4). It accounted for 61.8% (21/34) of all cancers reported, representing 57.7% (15/26) of all *SMAD4* and 87.5% (7/8) of all *BMPR1A* cancers. Eighty percent (12/15) of *SMAD4* variants were nonsense/frameshift type compared with 28.6% (2/7) in *BMPR1A* carriers ($p = 0.052$). Gastric cancer ($n = 7$; all *SMAD4*; median age at diagnosis: 44 years; range 38–55 years) was reported in *SMAD4* carriers only (5.5%; 7/127) and accounted for 20.6% (7/34) of all cancers reported and for 26.9% (7/26) of all *SMAD4* tumors. Similar observations were made in the LBSB group where colorectal and gastric cancer accounted for 63% ($n = 12$) and 15.8% ($n = 3$; all *SMAD4*) of the cancer burden. Few other cancers reported in the ECS group were located in the small intestine ($n = 1$), thyroid ($n = 2$), lung ($n = 1$), and cancer of unknown origin ($n = 1$).

Six (2.7%) ECS patients were reported to have developed metachronous cancers (Table 4). In three of them both cancers had occurred in the GI tract (three colorectal, three gastric, and one cancer of the small intestine), whereas three, each with colorectal cancer, had additionally developed lung, pancreatic, and thyroid cancer, respectively.

Pathogenic variant type frequencies

Overall, frameshift, nonsense, and missense variants accounted for the majority of pathogenic *SMAD4* (72.9%) and *BMPR1A* (61.8%) alterations in the ECS as well as the LBSB group (*SMAD4*: 79.9%; *BMPR1A*: 70.8%; Supplementary Table 3). Only large genomic, i.e., single or multiexon deletions in *SMAD4* were significantly overrepresented in the ECS compared with the LBSB group (21.4% vs. 6.8%; $p = 0.003$). De novo variant occurrence was suspected in 10 (7.9%; 8 *SMAD4*, 2 *BMPR1A*) of 126 ECS index patients as well as in 26 (5.5%; 11 *SMAD4*, 15 *BMPR1A*) of 473 LBSB pathogenic variant carriers.

As detailed above, statistically significant genotype–phenotype correlations were identified with regard to younger age at diagnosis in carriers of 10q23 microdeletions encompassing the *BMPR1A* and *PTEN* loci. Interestingly, with regard to juvenile polyposis of infancy, the three index patients, aged between 7 months and 2 years at diagnosis, all carried *BMPR1A* PVs; only one, however, carried the 10q23 microdeletion while the other two harbored an exon 1 deletion and a splice site alteration (c.430+2T>C), respectively. In *SMAD4* PV carriers a more severe gastric phenotype as well as an HHT overlap phenotype were observed. In addition, index patients with *SMAD4* missense PVs ($n = 14$) displayed a significantly younger median age at diagnosis (10.5 years, IQR 22.0) compared with those with *SMAD4* frameshift changes (28.0 years, IQR 31.0, $n = 29$; $p = 0.028$); this finding, however, was not present among nonindex patients (missense: 36 years, IQR 12.8; frameshift: 29 years, IQR 30.0; $p = 0.39$). Even when combining the ECS and LBSB data sets no further statistically significant phenotypic differences with regard to the type of genetic variant or protein domain affected could be identified. We assume that this is partly due to the comparatively small numbers in the respective variant subgroups and partly to the pronounced phenotypic heterogeneity in JPS. The latter was particularly striking in carriers of identical germline variants (e.g., 19 patients from 9 unrelated families carrying the c.1244_1247del *SMAD4* variant). Moreover, marked intrafamilial phenotypic heterogeneity, in particular regarding JP and cancer occurrence, was evident in four (1 *SMAD4*, 3 *BMPR1A*)

Table 5 Surveillance recommendations in juvenile polyposis.

Organ	Patients	Methods	Age at starting	Interval
Colon, Rectum	All JPS patients	Colonoscopy	12–15 years	1–3 years Depending on phenotype
Stomach, Duodenum	*SMAD4*	Gastroduodenoscopy	18 years	1–3 years Depending on phenotype
	BMPR1A		25 years	3 years
Vascular manifestations	*SMAD4*	Referral to HHT specialist Individual screening		

HHT hereditary hemorrhagic telangiectasia, *JPS* juvenile polyposis syndrome.

ECS families with data available on five to ten family members (data not shown).

DISCUSSION

This large comprehensive retrospective study on genotype–phenotype correlations in JPS corroborates previous observations that *SMAD4* carrier status is associated with HHT, multiple gastric JPs, and gastric cancer occurrence; provides recommendations for management on the basis of both new and previously published data (Table 5); and establishes the basis for open access *SMAD4* and *BMPR1A* Leiden Open Variant Database–powered variant databases.[19]

The reported age at diagnosis of JPS was similar for both *SMAD4* and *BMPR1A* pathogenic variant carriers (median 28 and 25 years, respectively). The youngest index patients reported, both in the ECS and the LBSB group (median age 1.5 and 6 years, respectively), consisted of carriers of large cytogenetic alterations at 10q23, e.g., microdeletions encompassing the *BMPR1A* and *PTEN* loci.

As expected, JPS-HHT symptoms in both the ECS and LBSB group were exclusively reported in *SMAD4* but not in *BMPR1A* pathogenic variant carriers. In addition, anemia was twice as often observed in *SMAD4* carriers with 32% displaying the hemorrhagic telangiectasia overlap syndrome (HHT) phenotype ($p = 0.0006$). Unfortunately, the true incidence of HHT/JPS overlap in those with a *SMAD4* PV cannot be established, as not all *SMAD4* carriers underwent systematic screening for HHT. However, given that almost one third had features of HHT in this cohort, in addition to past publications suggesting very high (around 90%) prevalence of JPS/HHT overlap in *SMAD4* carriers,[9] we recommend that all patients with a *SMAD4* PV undergo routine screening for HHT, such as a one-off thoracic computed tomography (CT) scan to screen for pulmonary arteriovenous malformations and contrast echocardiogram for aortopathy. Surveillance guidelines are not uniform across countries, and thus referring such patients to a local HHT specialist center would be advised (European centers can be identified at https://vascern.eu/expertise/rare-diseases-wgs/hht-wg/).

While JPs of the colon were twice as frequently reported in ECS index patients compared with affected family members, rectal JPs were significantly less frequent than colonic ones (32% vs. 72%; $p < 0.01$), independent of the gene affected. The prevalence and number of gastric JPs, however, were significantly increased in both *SMAD4* index and nonindex patients, and occurred roughly 20 years later compared with the colonic JPs (40 vs. 18 years; $p < 0.0001$). Upper GI endoscopy surveillance is recommended for patients with JPS. The data presented here are sufficient, when combined with published data, to support that upper GI surveillance should be tailored to the underlying genotype. It would be reasonable to recommend upper GI surveillance to start at age 18 years for *SMAD4* carriers, with intervals ranging from 1 to 3 years depending on the number of polyps (Table 5). However, although approximately 18% of *BMPR1A* carriers develop gastric polyps, they are usually smaller and gastric cancer has not been reported. In view of these findings, starting surveillance in this group by the age of 25 years and a surveillance interval of 3 years seems reasonable until further data are available.

Among the 11% to 15% of LBSB and ECS patients with cancer, colorectal cancer accounted for 58% (*SMAD4*) and 88% (*BMPR1A*) of all cancers reported and occurred about 20 years later (median age 41 years) compared with the age at diagnosis of colonic JPs (18.5 years; $p < 0.0001$). Most patients' colorectal disease is likely to be manageable endoscopically. Recent pediatric guidelines produced on behalf of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPHGAN) outline an approach to colorectal surveillance, commencing at age 12–15 with surveillance interval being personalized according to the individual's phenotype.[20] However, some patients will require surgical intervention for either endoscopically unmanageable disease, or the development of cancer. Where surgery is being contemplated, it should be personalized according to the individual's phenotype. Usually in the prophylactic setting, a (sub)total colectomy will be the procedure of choice, retaining the rectum, avoiding the need for pelvic dissection.

As with the prevalence of gastric JPs, gastric cancer (median age 44 years) was exclusively reported in *SMAD4* PV carriers, accounting for 27% of *SMAD4* tumors. These observations were essentially mirrored by the LBSB group. It is likely that gastric cancer occurrence in *SMAD4* carriers is underestimated since seven ECS patients with massive JP burden had prophylactic gastric resection/gastrectomy at a median

age of 49.5 years. Thus, more of these patients might have developed gastric cancer. It is difficult on the basis of current evidence to give firm recommendations regarding gastrectomy in *SMAD4* carriers. There are no data regarding outcomes from endoscopic polypectomy in the stomach. Decisions will need to be made on a case-by-case basis. Those with advanced gastric polyposis are best seen in a center experienced in managing this condition. It is likely that the gastric polyps have a relatively indolent progression. However, in those with advanced polyposis in whom dysplasia is found, and in those whose stomachs cannot be adequately/safely surveyed, a discussion through a specialist multidisciplinary team is required and then the option of prophylactic/risk-reducing surgery should be discussed with the patient. As always, in the setting of prophylactic, risk-reducing surgery, retaining the best possible quality of life is essential and therefore this surgery should be reserved to highly specialized centers.

In this study small intestine polyps were reported in 15.7% (20/127) of *SMAD4* carriers and 3.2% (3/94) of *BMPR1A* carriers. It should be noted that we did not distinguish duodenal from ileal/jejunal polyps and it is likely that duodenal polyps predominate here.[21] In addition, given that small bowel surveillance is not routinely recommended, the true incidence of small bowel polyps cannot be established. However, these data do support the recommendation that the small bowel should undergo evaluation for patients who develop symptoms or unexplained anemia, e.g., with video-capsule endoscopy (VCE), to look for underlying small bowel polyps and angioectasia.

The limitations of this study obviously include its retrospective nature and, inevitably when dealing with such a rare disorder, the possibility of ascertainment and/or selection bias. In addition, differences in patient data collection as well as completeness of medical records among the participating centers will impact on data quality and comparability. Furthermore, certain of the reported phenotypic features likely represent underestimations since a particular trait may go unnoticed if not specifically searched for, e.g., arteriovenous malformations in *SMAD4*-associated JPS-HHT, as reported by O'Malley et al.[9]

To circumvent these issues clearly, prospective, multicenter-based studies, as exemplified by the Prospective Lynch Syndrome Database (PLSD), are needed,[22] which could also inform the outcomes of surveillance measures taken.[23] Furthermore, with the novel genetic high-throughput sequencing tools available, the roughly 50% of JPS patients and families in whom no *SMAD4* or *BMPR1A* alteration can be identified constitute an obvious target for candidate gene analysis.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-0826-1) contains supplementary material, which is available to authorized users.

ACKNOWLEDGEMENTS

We thank the patients and their families for participation in the study and Sophie Grandjouan for contributing clinical data. D.G.E. is supported through the National Institute for Health Research (NIHR) Manchester Biomedical Research Center (IS-BRC-1215–20007). K.H. is supported by grants from the Stiftung zur Krebsbekaempfung (number 285) and the Freiwillige Akademische Gesellschaft.

DISCLOSURE

The authors declare no conflicts of interest.

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