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Histologic heterogeneity and syndromic associations of non-ampullary duodenal polyps and superficial mucosal lesions

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ABSTRACT

Background: Duodenal polyps and superficial mucosal lesions (DP/SMLs) are poorly characterised.
Aims: To describe a series of endoscopically-diagnosed extra-ampullary DPs/SMLs.
Methods: This is a retrospective study conducted in a tertiary referral Endoscopy Unit, including patients who had DPs or SMLs that were biopsied or removed in 2010–2019. Age, gender, history of familial polyposis syndromes, DP/SML characteristics were recorded. Histopathological, immunohistochemical and molecular analyses were performed.
Results: 399 non-ampullary DP/SMLs from 345 patients (60.6% males; median age 67 years) were identified. Gastric foveolar metaplasia represented the most frequent histotype (193 cases, 48.4%), followed by duodenal adenomas (DAs; 77 cases, 19.3%). Most DAs (median size 6 mm) were sessile (Paris Is; 48%), intestinal-type (96.1%) with low-grade dysplasia (93.5%). Among syndromic DAs (23%), 15 lesions occurred in familial adenomatous polyposis 1, two were in *MUTYH*-associated polyposis and one was in Peutz-Jeghers syndrome (foveolar-type, p53-positive, low-grade dysplasia). Only one (3.3%) tubular, low-grade DA showed mismatch repair deficiency (combined loss of *MLH1* and *PMS2*, heterogeneous *MSH6* expression), and it was associated with a *MLH1* gene germline mutation (Lynch syndrome).
Conclusion: DPs/SMLs are heterogeneous lesions, most of which showing foveolar metaplasia, followed by low-grade, intestinal-type, non-syndromic DAs. MMR-d testing may identify cases associated with Lynch syndrome.

1. Introduction

Duodenal polyps and superficial mucosal lesions (DP/SMLs) are relatively uncommon findings, with an estimated prevalence of 1–5% in patients undergoing esophagogastroduodenoscopy (EGDS) [1–3] Indeed, most DP/SMLs are asymptomatic and are incidentally found. When clinically relevant, DP/SML-related symptoms may include dyspepsia, abdominal pain, and intestinal bleeding.

Histologic evaluation is pivotal to determine the neoplastic or non-neoplastic nature of DP/SMLs and to predict its malignant potential [4,5]. A wide spectrum of histologic entities may appear endoscopically as a DP/SML, ranging from benign non-neoplastic lesions to overt neoplastic lesions [6]. Importantly, different DP/SML histotypes may require different therapeutic options [7]. Among duodenal epithelial neoplasms, the precursor non-invasive lesions are termed adenomas, while their invasive counterparts are represented by adenocarcinomas and neuroendocrine tumours [8].

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The prevalence of the different histologic entities among DP/SMLs has been poorly investigated. Also, little is known about the endoscopic and histologic features of duodenal adenomas (DAs), and most studies on this topic are from Japanese groups [9–12]. Duodenal epithelial neoplastic lesions may arise sporadically or in a background of genetic polyposis syndromes, such as familial adenomatous polyposis 1 (FAP), *MUTYH*-associated polyposis (MAP), Peutz-Jeghers syndrome, and juvenile polyposis syndrome [8]. Moreover, Lynch syndrome (LS) is associated with an increased risk of both colorectal and duodenal adenocarcinomas, thus, testing small and large intestinal carcinomas for mismatch repair deficiency (MMR-d) is now recommended, also considering that the small bowel is likely the gastrointestinal organ with the highest proportion of MMR-d cancers [13]. The presence of MMR-d in colorectal adenomas proved to be rare, but highly specific for LS [14]; however, little is known about its significance in DAs.

In the present study, we aimed to analyze a series of extra-ampullary DPs/SMLs at a single Italian tertiary referral center over a ten-year period and to report the prevalence of the various histopathologic entities. Moreover, we compared the clinicopathological features of syndromic and non-syndromic DAs. Finally, we aimed to investigate the MMR-d frequency in non-syndromic DAs and its association with LS.

2. Material and methods

2.1. Study population

The electronic medical database of the Digestive Endoscopy Unit of San Matteo Hospital Foundation, Pavia, Italy, was retrospectively examined in the period January 2010–December 2019. We searched the following medical heading terms “duodenal polyp”, “duodenal mucosal lesion”, or “indeterminate duodenal neoformation”. Lesions of the ampulla of Vater and endoscopic exams without subsequent specimen histological evaluation were excluded. DP/SMLs which were first biopsied, and then either resected or re-biopsied, were counted only once. Only adult patients were included in the study. The study was approved by the Local Ethical Committee.

2.2. Demographic, endoscopic and histologic features

Data regarding patient age, gender, personal or family history of any familial polyposis syndrome, DP/SML location and size, and type of resection (through biopsy forceps, snare polypectomy, or endoscopic mucosal resection (EMR), “en bloc” or “piecemeal”) were recorded. Biopsy forceps were used only for lesions < 2 mm, while either snare polypectomy or EMR were used for bigger lesions. All patients underwent EGDS (Olympus gastroscopes), performed by an experienced endoscopist. Endoscopically obtained specimens were sent to the Anatomical Pathology laboratory and fixed in 4% buffered formalin for 12–18 h and then embedded in paraffin. For each specimen, 4-μm-sections at different levels were obtained and stained with haematoxylin/eosin (HE) and Alcian blue/periodic acid-Schiff (AB-PAS).

DP/SMLs were classified histologically into the following categories according to established criteria [6]: gastric foveolar metaplasia (consisting of gastric foveolar metaplastic cells without oxyntic glands), gastric heterotopia (gastric foveolar cells and oxyntic glands), inflammatory polyps (inflammatory proliferation of the duodenal mucosa, often composed of granulation tissue), Brunner’s gland proliferative (hyperplastic/hamartomatous) lesions (characterized by hyperplastic, histologically normal Brunner glands), hamartomatous polyp (a growth composed of an abnormal mixture of tissues normally found in the duodenum), hyperplastic polyp (characterized by columnar microvesicular epithelium with luminal serration and without cytologic atypia), adenoma (dysplastic epithelium/non-invasive epithelial neoplasia), adenocarcinoma (invasive glandular neoplasia), neuroendocrine tumor (NET), non-epithelial neoplasia (including mesenchymal and lymphoid neoplasms), metastasis, lymphangiectasia and “non-significant histologic alterations”. Brunner gland proliferative lesions were differentiated from pyloric gland adenomas because lobular architecture was maintained in the former and at least partly lost in the latter; foveolar metaplasia was distinguished from foveolar-type adenomas by the absence of unequivocally dysplastic features and surface maturation in the former; foveolar metaplasia cases showing mild, equivocal epithelial nuclear atypia were labelled as “atypical foveolar metaplasia” [1].

All DA were regarded as premalignant lesions consisting of dysplastic epithelium, according to the criteria of the World Health Organization (WHO) classification of tumours of the digestive system [8]. DAs were further subdivided clinically into syndromic cases (i.e., those occurring in the setting of a clinically and genetically confirmed familial polyposis syndrome) and non-syndromic cases (i.e., those arising in patients without a personal or family history of polyposis syndromes and without evidence of a gastrointestinal polyposis by EGDS and colonoscopy). DA morphologic shape was determined according to Paris endoscopic classification [15], while their size was measured by the endoscopist. Histologically, they were classified into two subtypes according to their histologic differentiation phenotype, i.e., intestinal-type DA and gastric-type DA [8]. Moreover, all DAs were morphologically subdivided into tubular or tubulovillous according to their architecture and classified as low grade or high grade. High grade DAs were defined by prominent architectural complexity (cribriform or back-to-back glands), marked nuclear stratification and severe cytological atypia [8].

Histologic analysis of all tissue sections was performed by three pathologists (RC, AV, OL); in case of discordance between pathologists, histologic slides were re-reviewed, until a consensus was reached.

Table 1
Antibodies used for immunohistochemistry.

Marker	Clone	Manufacturer	Dilution
MLH1	ES05	Dako	Pre-diluted
MSH2	FE11	Dako	Pre-diluted
MSH6	EP49	Dako	Pre-diluted
PMS2	EP51	Dako	Pre-diluted
p53	DO-7	Dako	Pre-diluted
MUC5AC	CLH 2	Abcam	Pre-diluted
MUC6	CLH 5	Novocastra	Pre-diluted

2.3. Immunohistochemistry

Antibodies used for immunohistochemistry were listed in Table 1.

Gastric-type DAs were further subclassified into “foveolar adenoma” or “pyloric gland adenoma”, according to the same morphologic and immunohistochemical criteria used for classification of gastric adenomas [6,9,16]. Pyloric gland adenomas were composed of tightly packed glands lined by a single layer of cuboidal or low columnar cells, with a granular, eosinophilic-to-ground-glass cytoplasm and round nucleus, resembling pyloric glands, coupled with diffuse expression of MUC6 and selective expression of MUC5AC along the surface epithelium, while foveolar adenomas were composed by MUC5AC-positive tall columnar dysplastic cells with a typical apical mucin cap, resembling gastric foveolar cells, with only rare MUC6 positive cells. In selected cases, p53 immunohistochemistry was performed in order to confirm the dysplastic nature

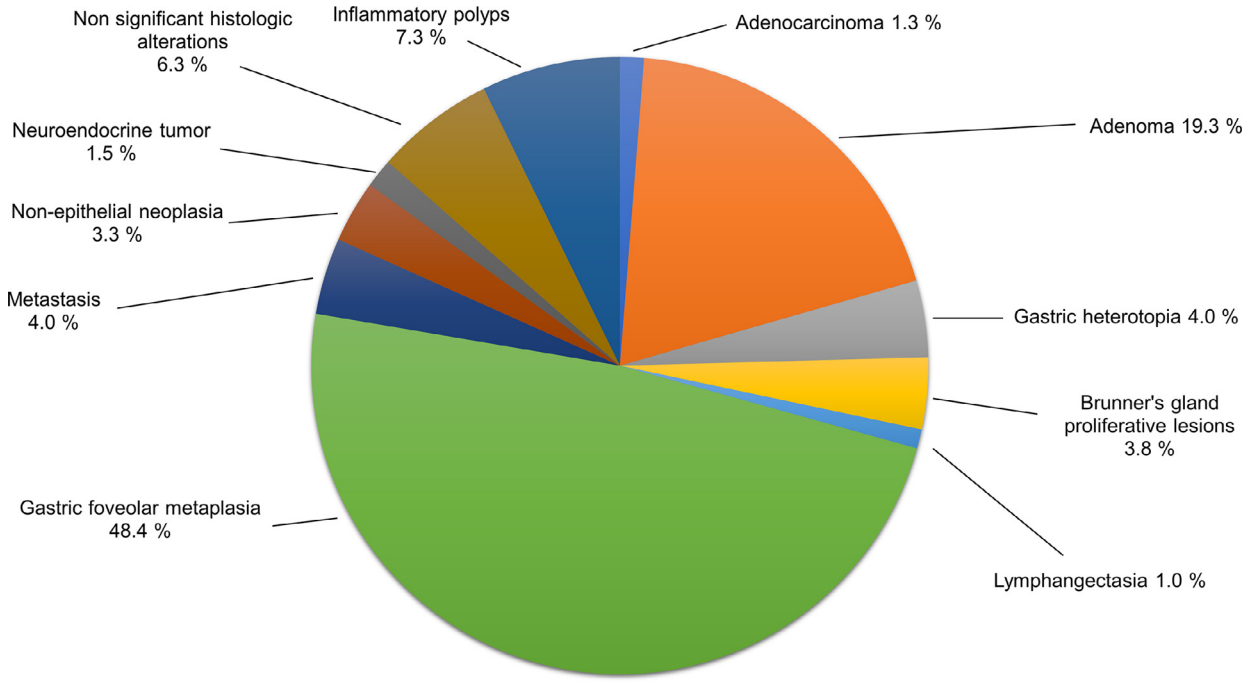

Fig. 1. Prevalence of different histologic categories among non-ampullary duodenal polyps/superficial mucosal lesions (period 2010–2019).

Table 2
Endoscopic and histologic features of syndromic and non-syndromic duodenal adenomas.

		Total	Syndromic	Non-syndromic
N (%)		77 (100)	18 (23)	59 (76)
Adenoma size median (IQR), mm		6 (4–12)	5.5 (3.6–8.5)	6 (4–13)
Paris classification, N (%)				
	Ip	2 (2.6)	0 (0)	2 (3.4)
	Is	37 (48)	12 (66.7)	25 (42.4)
	IIa	30 (39)	5 (27.8)	25 (42.4)
	IIa-IIc	5 (6.5)	1 (5.5)	4 (6.8)
	IIc	3 (3.9)	0 (0)	3 (5)
Endoscopic procedure, N (%)				
	Polypectomy	29 (37.7)	7 (38.8)	22 (37.3)
	EMR	17 (22)	2 (11.2)	15 (25.4)
	Biopsy	31 (40.3)	9 (50)	22 (37.3)
Histologic phenotype, N (%)				
	Intestinal-type	74 (96.1)	17 (94.5)	57 (96.6)
	Gastric- type	3 (3.9)	1 (5.5)	2 (3.4)
Histologic architecture, N (%)				
	Tubular	55 (71.5)	14 (77.8)	41 (69.5)
	Tubulovillous	22 (28.5)	4 (22.2)	18 (30.5)
Grade of dysplasia, N (%)				
	Low grade	72 (93.5)	17 (94.5)	55 (93.2)
	High grade	5 (6.5)	1 (5.5)	4 (6.8)

Abbreviations: EMR, endoscopic mucosal resection; IQR, interquartile range.

of the lesion; a positive result for p53 was defined as the presence of strong nuclear staining in > 50% of the lesional cells.

Non-syndromic DAs with available tissue sections were also examined for the expression of the MMR proteins MLH1, PMS2, MSH2 and MSH6 by immunohistochemistry (Table 1). DAs were considered as MMR-proficient (MMR-p), if nuclear expression of all MMR proteins was retained, or MMR-deficient (MMR-d) if nuclear staining of at least one MMR protein was absent in at least 10% of adenomatous cells, in the presence of an internal positive control, represented by stromal or inflammatory cells or non-tumor mucosa.

2.4. Molecular analyses

DAs with loss of expression of MMR proteins (i.e., MMR-deficient DA) were tested for microsatellite instability (MSI). Tumor DNA from each patient was obtained from formalin-fixed and paraffin-embedded tissues using three representative 8 μm-thick sections of tumor samples. DNA was extracted using an automatic nucleic acid purification system (Maxwell® 16 system, Madison, Wisconsin, USA). MSI analysis was carried out using a pentaplex panel of monomorphic mononucleotide repeats (*BAT25*, *BAT26*, *NR-21*, *NR-22*, and *NR-24*) as previously reported [17]. We analysed five regions of *MLH1* promoter encompassing -658 to +206 positions with respect to ATG codon by MS-MLPA using ME011-C1 kit. Each sample was analysed in duplicate and a methylation ratio for each probe (MR) was calculated by Coffalyser software (version 140,721.1958) following the instructions of the manufacturer and performing data normalization within each experiment (MRC Holland, Amsterdam, The Netherlands).

Aberrant *MLH1* methylation was scored as a categorical variable using a methylation cut-off of 0.2 as previously reported [18]. *BRAF*

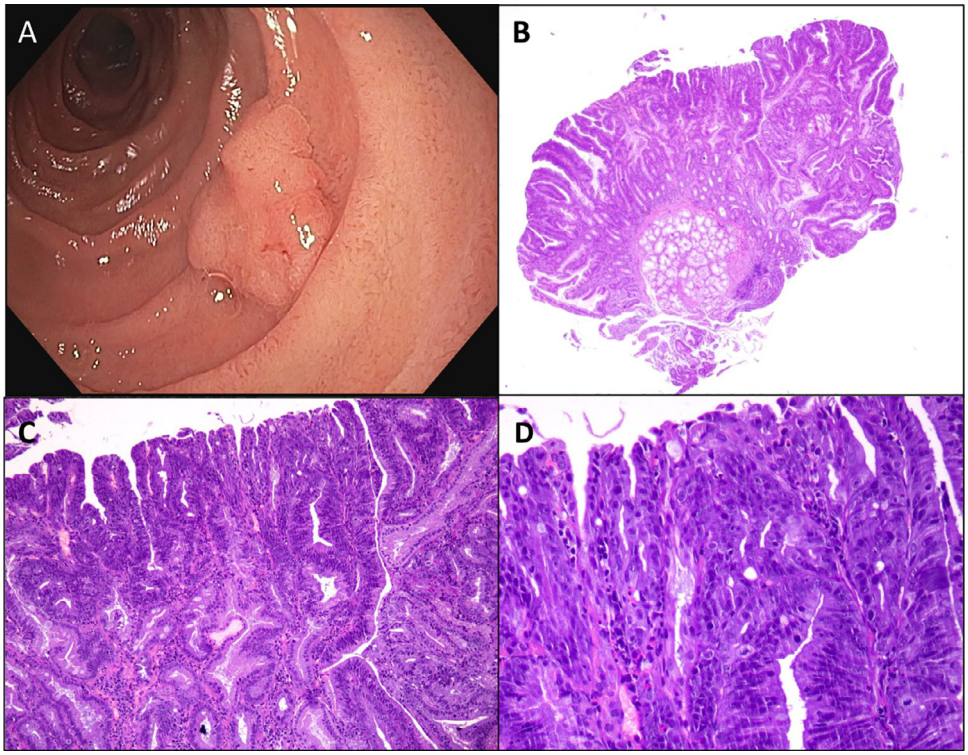

Fig. 2. Endoscopic (**A**; frontal view, white-light) and histologic images (**B–D**, haematoxylin and eosin) of an intestinal-type, high-grade duodenal adenoma in a *MUTYH*-associated polyposis (MAP) syndrome patient. The lesion appeared as a 15 mm sessile polyp (Paris 0-Is), with a superficial roundish pit pattern. Note in **D**, at higher magnification, the architectural complexity with back-to-back glands, coupled with marked nuclear stratification.

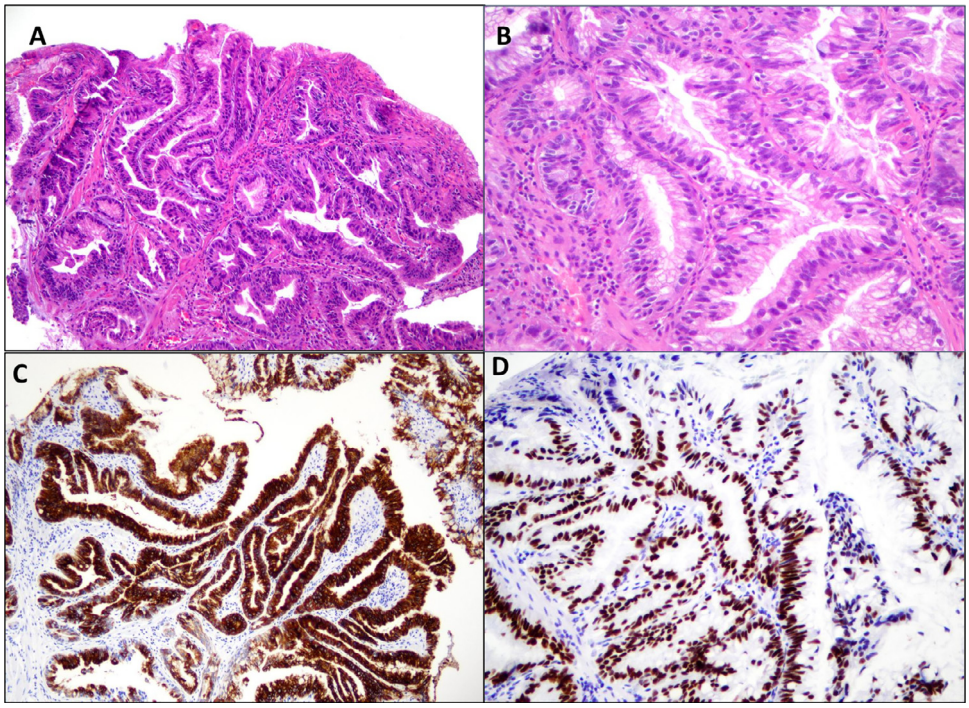

Fig. 3. Histologic images of a foveolar-type, low-grade duodenal adenoma arising in a Peutz-Jeghers patients, composed of tubulo-villous structures lined by tall columnar epithelial cells (**A, B,** haematoxylin and eosin), with a MUC5AC-positive apical mucin cap, resembling gastric foveolar cells (**C,** MUC5AC immunohistochemistry). Note the nuclear reactivity of dysplastic cells for p53 (**D**, p53 immunohistochemistry).

mutation analysis was performed using Myriapod Colon Status (Diatech Pharmacogenetics, Italy) on MALDI-TOF instrument (Agena BioscienceTM, Hamburg, Germany). The spectra were processed using SpectroACQUIRE software (Sequenom) and genotype calls were automatically generated using mathematical algorithms by the MassARRAY Typer 4.0 software (Sequenom). Dossier software (Diatech Pharmacogenetics) was used for automated data analysis, accompanied by visual inspection of extension products.

Whole-exome sequencing (WES) was carried out on genomic DNA extracted from peripheral blood with the Maxwell® 16 LEV Blood DNA Kit (Promega, Madison, WI). Whole-exome enrichment was performed in outsourcing using the Twist Human Core Exome

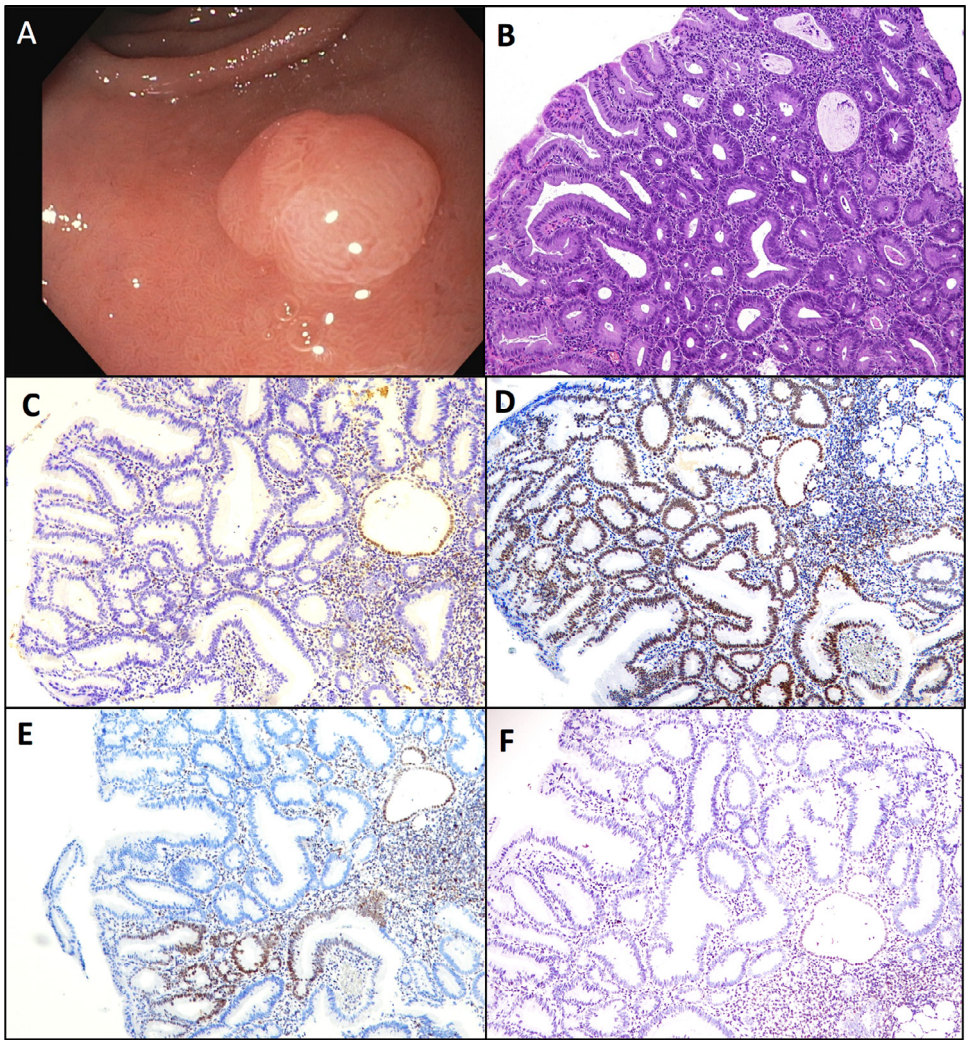

Fig. 4. Endoscopic (**A**; white-light, front view) and histologic images (**B–D**) of a case of Lynch-syndrome associated intestinal-type, low-grade duodenal adenoma (**B**, haematoxylin and eosin), showing combined loss of expression of MLH1 (**C,** MLH1 immunohistochemistry) and PMS2 (**F**, PMS2 immunohistochemistry), and heterogeneous expression of MSH6 (**E**, MSH6 immunohistochemistry) by the adenomatous cells, while MSH2 expression was retained in all adenomatous nuclei (**D**, MSH2 immunohistochemistry). Note the nuclear reactivity of normal intestinal crypt cells and inflammatory and stromal lamina propria cells for all mismatch repair proteins, as internal positive controls. The lesion appeared as a 6 mm sessile polyp (Paris 0-Is), with a superficial tubular pit pattern.

Kit (Twist Bioscience, San Francisco, CA) on a NovaSeq 6000 platform (Illumina, San Diego, CA).

2.5. Statistical analysis

Our primary aim was to describe the histological, immunohistochemical and molecular characteristics of DP/SMLs. As a secondary aim, we looked at possible differences between non-syndromic and syndromic lesions. Data were described with the median and interquartile range if continuous and with counts and percentages if categorical. Mann-Whitney and Fisher's exact tests were used for comparing the variables of interest. All tests were two-sided, and statistical significance was set at $p < 0.05$. The software Stata 14.1 [StataCorp., College Station, TX] was used for computation.

3. Results

3.1. Prevalence of the different histologic lesions among DP/SMLs

Over the study period, 32,674 EGDS were performed (mean of 3267 per year). We identified 399 non-ampullary DP/SMLs from 345 patients, with a predominance of male patients (male to female ratio 1.65:1; median age 64 years (53–73). Hence, considering the number of patients affected by at least one lesion, we found a cumulative incidence of 0.1 cases per person-year. The distribution of the different histologic lesions among DP/SMLs is summarized in Fig. 1.

Gastric foveolar metaplasia of the duodenal surface epithelium represented the most frequent histotype of DP/SMLs (193 cases, 48.4%), followed by DAs (77 cases, 19.3%). This finding was confirmed in both bulbar and non-bulbar locations. In two DP/SMLs with gastric foveolar metaplasia a mild nuclear atypia was observed, despite surface maturation, seamless transition to the surrounding epithelium and absence of p53 reactivity, thus falling short of a diagnosis of dysplasia/adenoma; they were regarded as atypical foveolar metaplasia. Among non-neoplastic lesions, a significant number of inflammatory polyps, often composed of granulation tissue, were also identified (29 cases, 7.3%), three of which occurred in coeliac disease patients, while one of which arose in a background of graft versus host disease (GVHD). Brunner gland proliferative lesions, gastric heterotopias, and lymphangiectasia accounted for only 3.8, 4, and 1% of all DP/SMLs, respectively. No case of hyperplastic polyp resembling the colorectal counterpart was seen.

Neoplastic DP/SMLs (117 cases, 29.3%) encompassed a heterogeneous spectrum of epithelial and non-epithelial lesions. In addition to DAs, epithelial neoplasms included duodenal adenocarcinomas (5 cases, 1.3%), metastatic carcinomas (16 cases, 4%), and neuroendocrine tumours (6 cases, 1.5%), while non-epithelial neoplasms (13 cases, 3.3%) comprised 8 lymphoproliferative diseases, one lipoma, one Kaposi sarcoma, two gastrointestinal stromal tumours and one melanoma. In 6.3% of DP/SMLs, no significant histologic alterations were identified.

3.2. Characterization of syndromic and non-syndromic DAs

The endoscopic and histologic features of DAs are outlined in Table 2. Among the 77 non-ampullary DAs, 18 (23.4%) developed in 9 patients with polyposis syndromes (median age: 47 years, 41–53 male to female ratio: 1.25: 1, including 6 FAP patients, 2 MAP patients and 1 Peutz-Jeghers patient) whereas 59 DAs (76.6%) from 53 patients (median age: 73 years, 62–80, male to female ratio: 1.12:1) were non-syndromic; four patients without familial polyposis syndromes developed more than one (range 2-3) DAs.

Overall DA size ranged from 1.5 to 30 mm, with a median of 6 mm. The endoscopic shape, according to Paris endoscopic classification, was Is in 37 cases (48%), IIa in 30 cases (39%), IIa-IIc in 5 cases (6.5%), IIc in 3 cases (3.9%) and Ip in only 2 cases (2.6%). Histologically, the vast majority of DAs were of intestinal-type (96.1%), whilst only three lesions (3.9%) were diagnosed as gastric-type, including two foveolar adenomas and one pyloric gland adenoma. No serrated adenomas or lesions were found. Most lesions (93.5%) exhibited a tubular architecture and low-grade dysplasia (93.5%); only 5 cases (6.5%) showed high grade dysplasia. High grade adenomas showed a higher median size and a higher rate of gastric differentiation in comparison with low grade adenomas (15 mm vs 6 mm and 20% vs 3%, respectively), although the differences did not reach statistical significance. No significant endoscopic or histologic differences between syndromic DAs and non-syndromic DAs were observed (Table 2). However, "Is" endoscopic shape was more frequent in syndromic DAs in comparison with non-syndromic cases, where Is and IIa shapes were equally represented.

Among syndromic DAs, 15 lesions occurred in the setting of FAP, 2 were MAP-associated and one was found in a patient with Peutz-Jeghers syndrome. All FAP-associated DAs were intestinal-type and low grade, while one (15 mm in size) of two MAP-associated adenomas (both intestinal type) was high grade (Fig. 2**)**. Interestingly, the Peutz-Jeghers patient showed a duodenal polyp with histologic characteristics of Peutz-Jeghers polyp associated with a foveolar-type (MUC5AC+), p53-positive, low-grade dysplasia; therefore, it was finally classified as a foveolar-type adenoma arising in a Peutz-Jeghers polyp (Fig. 3**)**.

3.3. Mismatch repair status of non-syndromic DAs

Thirty DAs not associated with polyposis syndrome from 30 patients (median patient age: 67.8 years) were tested for MMR proteins. All tested DAs were intestinal type (77% tubular, 23% tubulo-villous) and most of them (90%) were low-grade. Only one (3.3%) tubular, low-grade DA showed MMR-d (combined loss of *MLH1* and *PMS2* and heterogeneous *MSH6* expression; Fig. 4**)** and MSI-high, without MLH1 promoter methylation or *BRAF* mutation. It was found in a 65-year-old woman with a past history of ovarian carcinoma, which had not been tested for MMR-d. WES analysis on germline DNA revealed a heterozygous missense variant in *MLH1* [(NM_000249.4:c.2041G>*A*, p.(Ala681Thr)]. This substitution was classified as pathogenic according to the ACMG/AMP guidelines [19], thus confirming a diagnosis of LS. The patient developed another DA during follow-up; however, it was MMR-proficient.

4. Discussion

We herein highlighted a wide histologic heterogeneity of non-ampullary DP/SMLs and gastric foveolar metaplasia represented the most frequently encountered histotype of DP/SMLs (48.4%), followed by DAs (19.3%), the vast majority of which were non-syndromic and intestinal-type. In a Korean evaluation of non-ampullary DP/SMLs, inflammatory polyps were the most represented lesions [20], while they accounted for only 7% of DPs/SMLs in our series. We found fewer cases of Brunner's gland proliferative lesions and no case of hyperplastic polyp, which is a very uncommon duodenal lesion, contrasting the high frequency in the large bowel [21].

Foveolar metaplasia is a common finding in duodenal biopsy specimens, especially in the duodenal bulb, as a reactive process after ulcers or chronic inflammatory diseases [22]. Two cases of foveolar metaplasia in our series were classified as "atypical foveolar metaplasia", a concept that is still debated. Sakurai et al. described a few cases of foveolar metaplasia around dysplastic foci, showing a gradual increase in nuclear atypia in the transition from metaplastic to dysplastic gland: this may represent a continuous spectrum in carcinogenesis from foveolar hyperplasia through atypical metaplasia or dysplasia, to overt adenocarcinoma [23]. Interestingly, a Japanese group found recurrent *GNAS* and/or *KRAS* mutations in a significant proportion of duodenal foveolar metaplasia, implying a potential role of these genetic alterations in the development of these lesions; moreover, recurrent *GNAS* mutations were also found in pyloric gland adenomas and gastric-type duodenal adenocarcinomas, suggesting that foveolar metaplasia may be a potential precursors of gastric-type duodenal neoplasms [24,25]. In addition to foveolar metaplasia, gastric heterotopia and Brunner gland proliferative lesions have been suggested as possible precursors of duodenal pyloric gland adenomas and non-ampullary duodenal adenocarcinomas [1,26,27].

DAs accounted for the majority of neoplastic DP/SMLs (77/117, 66%). To our knowledge, there is only another study evaluating the frequency of DA among overall non-ampullary DP/SMLs [20]. In contrast to Jung et al., our results showed a higher frequency of DA (19.3%). Endoscopically, the "Is shape" was the most frequent (48%), followed by "IIa type" (39%). Histologically, the vast majority of them were of intestinal-type (96%), confirming previous reports [9]**.** Among the rarer gastric-type DAs, we observed two foveolar adenomas, exceedingly rare and poorly known lesions in the duodenum, and one pyloric gland adenoma, a likely less rare lesion characterized by proximal location and a relatively high, albeit variable, rate of progression to invasive carcinoma (10–66%), but a low rate of recurrence after complete endoscopic resection [1,28].

We confirm that endoscopic and histologic features of syndromic DAs are similar to those of their non-syndromic counterparts. Although syndromic adenomas seem to be more common than sporadic adenomas in the non-ampullary duodenum [29], in our series non-syndromic adenomas (76%) were more frequent, in keeping with previous findings [30]. While the high frequency of syndromic DAs in some reports may have been overestimated due to a selection bias, a limit of our study could be that we did not perform genetic analysis in all patients with DAs. However, in the absence of clinical diagnostic criteria for gastrointestinal polyposis syndrome or of an MMR-d phenotype, we expect that the risk of an underlying genetic tumor syndrome is very low.

A fraction of DA may have the potential to progress to adenocarcinoma according to an adenoma-carcinoma sequence, similar to their colo-rectal counterpart [5,31], even though a recent integrated genetic and epigenetic study suggests that such a sequence

plays a limited role in duodenal carcinogenesis [32]. At the present, it remains difficult to distinguish DAs that could be followed up from those that require resection, Okada et al. reported that high grade dysplasia and DA size of 20 mm or larger are the two major risk factors for neoplastic progression [4]. Thus, DA size and grade help guide therapeutic decisions. Given the low risk of progression to adenocarcinoma (about 5%), low-grade DA can be followed-up or treated endoscopically, while high grade DAs have a 55% risk of progression to adenocarcinoma and should be removed by EMR or surgical resection [1,5,6]. A lower proportion of high-grade DA was seen in our study (6.5%) in comparison with that reported in the Japanese literature (40–60%) [10,11]. The most plausible explanation is that Western and Eastern pathologists have been used for years different criteria for the graduation of dysplasia [33,34]. Also, this discrepancy may be partly explained by differences in study populations, by inclusions of intramucosal adenocarcinomas and/or surgically treated DAs in some series, and by different percentages of patients only followed up with biopsies, that might underestimate the grade of dysplasia [12]. Interestingly, among the five high grade DAs of our series, one (20% of high-grade vs 3% of low-grade adenomas) exhibited a gastric-type phenotype with predominantly foveolar differentiation, and it was the only one larger than 20 mm. Indeed, several studies observed that high grade dysplasia is associated with gastric-type differentiation in DAs [9].

The higher rate of high-grade dysplasia in MAP-associated DAs in comparison with FAP-associated DAs supports previous observations of a different molecular biology (e.g., a higher burden of somatic mutations in MAP-associated DAs) and natural history of DAs in FAP and MAP [35–38]. To note, despite the lower incidence of DAs in MAP compared to FAP, recent evidence suggests that duodenal adenocarcinomas in MAP may develop in the absence of advanced Spigelman stage or even in the absence of coexisting DAs, challenging the assumption that the same surveillance should be applied in both polyposis syndromes. In our series, all DAs in FAP and MAP were intestinal-type, while in a patient with Peutz-Jeghers syndrome we observed a duodenal polyp showing foveolar-type dysplasia, similar to that recently described in gastric polyps of another Peutz-Jeghers patient [39]. This finding is intriguing because it indicates that dysplasia in Peutz-Jeghers polyps, an exceedingly rare finding, may show a non-conventional phenotype at least in the upper gastrointestinal tract.

Besides polyposis syndromes, it is known that LS carriers, especially those with *MLH1* gene mutations, harbor a significantly increased risk of developing MMR-deficient duodenal adenocarcinomas [8]. Recent findings indicate that most LS-associated colorectal adenocarcinoma are preceded by MMR-deficient adenomatous phase, which can develop from MMR-d crypts or, more rarely, from a MMR-p adenoma by secondary MMR inactivation [40]. Similar molecular pathways may play a role also in the carcinogenesis of duodenal adenocarcinoma in LS patients. In addition, it was found that MMR-d in colorectal adenomas is highly specific for LS [14]. Literature data suggest that MMR-d/microsatellite instability is rare in DAs [32]. We decided to test non-syndromic DAs for MMR proteins by immunohistochemistry in order to identify possible LS patients. Surprisingly, we encountered one case (3.3%) of DA with complete loss of *MLH1* and *PMS2* expression and heterogeneous expression of *MSH6* in the adenomatous cell. Molecular analysis revealed that it was MSI-high, associated with a heterozygous pathogenic germline variant in *MLH1* gene, indicative of LS. Current screening guidelines for bowel cancer restrict MMR/MSI testing on bowel cancer, excluding precursor lesions from this analysis [41]. While this approach seems reasonable for colorectal adenomas, our results suggest to include the rarer DAs in MMR evaluation in order to identify unknown LS patients, especially when the patient has a history of a previous neoplasm. Indeed, a study of cost-effectiveness of including DA in MSI screening would be mandatory to determine the feasibility of such a proposal.

In conclusion, our results indicate that DPs/SMLs are characterized by a high histologic heterogeneity; the most frequent histotype is represented by foveolar metaplasia, followed by DAs, most of which are low-grade, intestinal-type and non-syndromic. Beyond the well-known association with familial gastrointestinal polyposis syndromes, DAs may be rarely associated with LS, and MMR-d testing may help identify such cases.

Declaration of Competing Interest

None to disclose for all authors.

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