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A new phenotypic manifestation of familial adenomatous polyposis

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Abstract Familial adenomatous polyposis (FAP) is an autosomal dominant disease with hundreds of colorectal adenomas in teenagers and progression to colorectal cancer if colectomy is not performed. We investigated the association of two phenotypic manifestations-oral mucosal vascular density (OMVD) and oral mucosal reflectance (OMR)—with FAP and patients with multiple colorectal adenomas. Thirty-three patients with FAP from 29 unrelated pedigrees with APC gene mutation, 5 with multiple adenomas and no known gene mutations, and 50 population controls were evaluated for the two different manifestations utilizing a photographic/spectrophotometric system capturing images and reflectance at various wavelengths. Statistical analysis was performed with student *t* test and test performance characteristics were calculated. There were no significant differences in demographic variables between the FAP and control group. A significant difference in OMVD between FAP patients and controls was noted, $P < 0.001$. The sensitivity and specificity of oral mucosal vascular density for FAP was 91 and 90%, respectively. No association between this phenotypic manifestation and age or gender was found. All 5 patient with multiple polyps were positive for OMVD and the value was significantly higher than controls, $P = 0.002$. No significant difference was noted in OMR between the two patient groups and controls. OMVD is a new phenotypic manifestation in patients with FAP and also may identify those with multiple adenomas without known gene mutation.

Keywords Familial adenomatous polyposis · Oral mucosal vascular density · Oral mucosal reflectance

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Introduction

Familial adenomatous polyposis (FAP) is one of two well-described forms of hereditary colorectal cancer. FAP is an autosomal dominant syndrome with virtually 100% penetrance caused by germline mutation of the APC (Adenomatous Polyposis Coli) gene located at chromosome 5q21 [1–4]. This disorder is characterized by the development of hundreds of colorectal adenomas in adolescence [5]. Nearly all affected individuals will develop colorectal cancer by the 6th decade of life, if prophylactic colectomy is not performed [5].

Patients with FAP can have extraintestinal manifestations. These include benign soft tissue and bony tumors, desmoid tumors, and extraintestinal cancers [6]. Two of these lesions, occult radio-opaque jaw lesions [7] and

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congenital hypertrophy of the retinal pigment epithelium [8], have been associated with the development of familial adenomatous polyposis.

In 2003, De Felice et al. reported the occurrence of increased oral mucosal vascular network complexity in persons affected with hereditary nonpolyposis colorectal cancer (HNPCC) also known as Lynch syndrome—the other well-described form of hereditary colorectal cancer [9]. In a second publication, these investigators describe abnormalities in labial vestibular oral mucosal reflectance in HNPCC patients not found in the healthy controls [10], though others have not confirmed this finding [11].

This report investigates two phenotypic manifestations, oral mucosa vascular density (OMVD) and oral mucosal reflectance (OMR), in patients with familial adenomatous polyposis compared to healthy controls. Also, OMVD is evaluated in a subset of patients with multiple polyps and no recognized germline mutation.

Methods

Study population

Oral mucosal vascular density and oral mucosal reflectance analysis was performed on subjects with familial adenomatous polyposis recruited from the Johns Hopkins Hereditary Colorectal Cancer Registry. Patients were considered to have FAP if they had more than 100 colorectal adenomas identified by colonoscopy and/or had a deleterious germline APC gene mutation on genetic testing. All FAP subjects had or were related to a first degree relative with a deleterious APC gene mutation. Control subjects were individuals with no personal or family history of colorectal cancer or adenoma. In addition five patients with multiple polyps (cumulatively 20 or more colorectal adenomas) and no mutation found (NMF) in the APC or MYH gene mutation were evaluated. The study protocol was approved by the Johns Hopkins University Joint Committee on Clinical Investigations (institutional review board), and written informed consent was obtained from all subjects.

Measurement of oral mucosal vascular density and oral mucosal reflectance

A 2 $\times$ 2 cm area of the lower oral labial vestibular mucosa was imaged in each subject using a system with magnifying optics, a liquid crystal tunable filter, and a xenon light source coupled to a ring illuminator. For each subject, 40 images were collected at different tunable filter settings starting with a pass-band wavelength of 500 nm increasing in increments of 5–700 nm. The images collected were normalized by images of a white reflectance standard to eliminate the light source spectral dependence.

The oral mucosal vascular density was calculated from a 300 $\times$ 600 pixel portion of the main image manually selected by the operator who was blinded to the status of the subject. Each vessel in the image was traced using an automatic tracing algorithm by Sofka et al. [12]. A binary map of the traced vessels was generated and inputted into an algorithm quantifying the Kolmogorov Complexity of traced images and calculating an oral vascular density score for each subject [13]. The oral mucosal reflectance was also calculated from the same 300 $\times$ 600 pixel portion of the main image. The average value of all pixels was calculated, and this value corresponded to the total normal reflectance.

Statistical analysis

Analysis for differences in demographic characteristics between subjects with familial adenomatous polyposis and controls was done by a two-tailed unpaired Student's t test. The values for oral mucosal vascular density and oral mucosal reflectance in patients with familial adenomatous polyposis and five patients with multiple polyps and no mutation found were compared to those of healthy controls by Student's t test. A probability of $P < 0.05$ was considered statistically significant. Receiver operator characteristic (ROC) curves were used to determine the accuracy of oral mucosal vascular density and oral mucosal reflectance levels to discriminate between those affected and unaffected with FAP over a range of cut off points.

Results

Thirty-three patients with familial adenomatous polyposis from 29 different pedigrees with known deleterious APC gene mutations and 50 healthy controls without personal or family history of colorectal cancer or adenomas were evaluated. There were no significant differences in demographic characteristics between the two subject groups (Table 1).

Table 1 Demographic characteristics of the subjects

Characteristic	FAP (n = 33)	Controls (n = 50)
Age (year): Mean ± SD	42.7 ± 17.2	36.7 ± 17.6
Range	10–74	10–73
Sex: no. (%)		
Female	19 (58)	27 (54)
Male	14 (42)	23 (46)
Caucasian race: no. (%)	33 (100)	50 (100)

The mean oral mucosal vascular density was statistically significantly higher in those with FAP compared to controls (Table 2; Fig. 1a). Analysis revealed no association between oral mucosal vascular density and age or gender.

The sensitivity and specificity of oral mucosal vascular density to discriminate between individuals with familial adenomatous polyposis and controls was analyzed by receiver operator characteristic curve (Fig. 2). The area under the ROC curve was 0.91. An oral mucosal vascular density cut off level of 0.237 was 91% sensitive and 90% specific for the diagnosis of familial adenomatous polyposis.

In contrast, oral mucosal reflectance was not statistically significantly different between those with familial adenomatous polyposis and the control group over a series of wave spectrum from 500 to 750 um (Table 2; Fig. 1b).

The clinical characteristics of five patients with multiple polyps and no mutation found in the APC and or MYH gene are described in Table 3. Oral mucosal vascular density was statistically significantly higher in patients with multiple polyps and no mutation found (0.258 ± 0.017) compared to controls (0.219 ± 0.023), $P = 0.002$, but was not different from those with FAP, $P = 0.807$. All five patients in the NMF group had OMVD values greater than a cutoff level of 0.237, indicating they were positive for this phenotypic manifestation.

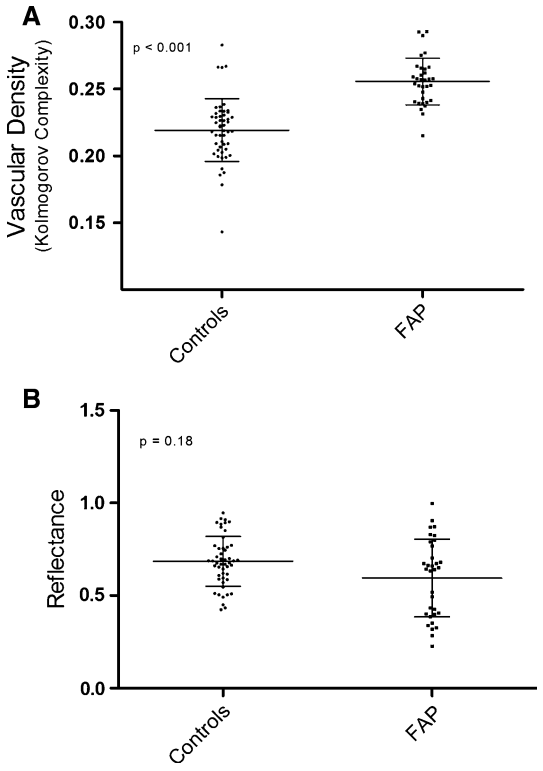

Fig. 1 *Scatter* plots showing the values for **a** oral mucosal vascular density and **b** oral mucosa reflectance for individuals with familial adenomatous polyposis (FAP) and controls

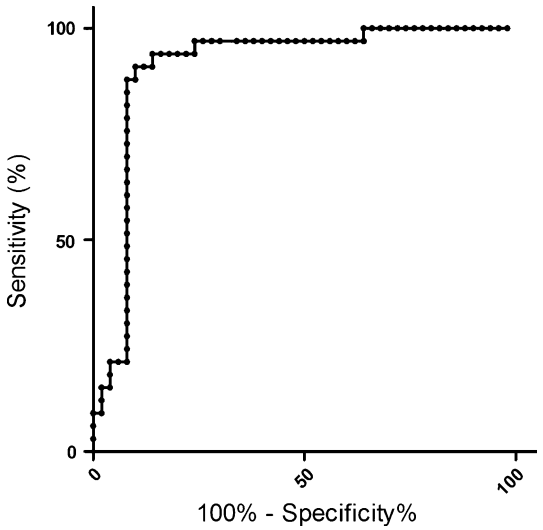

Fig. 2 Receiver operator characteristic curve for oral mucosal vascular density for familial adenomatous polyposis patients over a range of cutoff points

Discussion

In our study, patients with familial adenomatous polyposis had a statistically significantly increased oral mucosal vascular density compared to controls. This manifestation was not associated with age or gender. This is the first report, to our knowledge, of oral mucosal vascular density as a phenotypic manifestation in patients with familial adenomatous polyposis.

Moreover, OMVD was evaluated in a subset of patients with greater than 20 colorectal adenomas and no mutation found in the APC and MYH gene, the currently known causes of oligopolypsosis. In our study all 5 of these individuals had OMVD values greater than an established cut off. Also, the mean OMVD value in these patients was statistically higher than that of the controls but not different from FAP patients.

Several other phenotypic manifestations have been discovered in familial adenomatous polyposis patients. These include occult radio-opaque jaw lesions which are small,

Table 2 Oral mucosal vascular density and oral mucosal reflectance in FAP and control subjects

Parameter (mean ± SD)	FAP	Controls	*P* value
Oral mucosal vascular density	0.256 ± 0.017	0.219 ± 0.023	<0.001
Oral mucosal reflectance	0.633 ± 0.193	0.685 ± 0.135	0.175

Table 3 Patients with adenomatous polyposis or oligopolyposis and no mutation found in the APC and/or MYH gene

Patient	Age	Sex	Number of colorectal adenomas	APC testing	MYH testing	Family history
1	57	M	60	NMF[a]	NMF	Negative
2	43	M	20	NMF	NMF	Father young CRC (43)
3	50	M	30	NMF	NMF	Father multiple adenomas & CRC
4	53	M	25	NMF	NMF	Mother CRC (78)
5	50	M	>100	NMF		Mother CRC (74), sister with 40 polyps

[a] *NMF* no mutation found

usually multiple, well circumscribed radiodensities detected by panoramic X-rays in the premolar and molar regions of the mandible and maxilla [7]. A second phenotypic manifestation is congenital hypertrophy of the retinal pigment epithelium (CHRPE). These are discrete, round to oval, darkly pigmented retinal lesions ranging from 0.1 to 1.0 optic disc diameters in size detected by indirect ophthalmoscopy [8, 14].

In 2003, De Felice et al. first reported increased oral vascular network complexity in patients with hereditary nonpolyposis colorectal cancer the other well-described form of hereditary colorectal cancer [9]. In that report, members of a large Italian family with HNPCC (5 with mismatch repair gene mutation and 9 negative for mutation) were evaluated and compared to 30 unrelated sex-matched controls. Images from the lower gingival and vestibular oral mucosa from each subject were obtained using a hand held film camera, and subsequently, digitized for analysis using a film scanner. Analysis of the scanned images was performed by two operators who manually outlined the computerized two dimensional trajectories of the vascular network for each patient. This investigation found a statistically significantly increase in overall complexity and destructured randomness in the vascular networks of those with HNPCC compared to controls. In the present study, the usefulness of OMVD to identify patients with the other well-described form of hereditary colorectal cancer familial adenomatous polyposis was evaluated. In comparison to the methodology utilized by De Felice et al. the present study evaluated this marker in numerous FAP patients from multiple unrelated families. Also, the vascular network of each subject was directly captured by a high resolution digital optical sensor with analysis automatically performed by computer algorithm to enhance accuracy. In our study, OMVD was also associated with another hereditary form of colorectal cancer familial adenomatous polyposis.

In 2006, abnormal oral mucosal light reflectance in the oral gingival and vestibular oral mucosa was noted by De Felice et al., in patients with HNPCC from six affected families [10]. Oral mucosal color was assessed from photographic prints which were scanned by means of an imaging spectrophotometer. Our study analyzed the usefulness of this marker in FAP patients with direct measurement of the oral mucosa reflectance by spectrophotometer to eliminate the decrement of resolution and spectral color range which occurs when film is digitized and processed as paper prints. No abnormality of reflectance could be detected in FAP patients compared to controls. Also, Carrara et al., was unable to confirm abnormal light reflectance in 48 patients with HNPCC [11].

The mechanistic basis for increased oral mucosal vascular density in hereditary colorectal cancer is unknown. However, increased premalignant epithelial microvascular blood content is a common theme in neoplastic transformation. In the azoxymethane murine model of colorectal cancer, augmentation of colorectal superficial mucosal blood supply is noted preceding the appearance of mucosal aberrant crypt foci [15]. In normal human colonic mucosa, there is a threefold increase in superficial blood vessels in patients with advanced colorectal adenomas [15].

Analysis of microdissected human colon cancer tissue indicated a direct correlation between up regulation of VEGF-A expression and APC gene mutation status [16]. In addition, evaluation by immunohistochemistry of intestinal polyps in mice heterozygous for the multiple intestinal neoplasia gene (Min/+) (the murine model of human familial adenomatous polyposis) revealed an increase and redistribution of VEGF-A in the proximity of those cells expressing b-catenin with corresponding increase in vessel density [16]. Although these studies indicate a link between b-catenin signaling and the regulation of VEGF-A expression in colorectal mucosa, how noncolorectal tissue might be affected in those with perturbation of the Wnt pathway by germline APC mutation remains unclear.

In summary, the present study of familial adenomatous polyposis kindreds reveals that oral mucosal vascular density is a new phenotypic manifestation of the syndrome. Moreover, this manifestation appears to be present in patients with multiple colorectal adenomas and no recognized gene mutation. In contrast, oral mucosal reflectance was not associated with FAP. Future study of oral mucosal vascular density as a clinical marker of familial adenomatous polyposis and others at high risk for colorectal cancer is warranted.

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Conflict of interest None.

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