Chapter 12

The diagnostic value of mucocutaneous pigmentation in Peutz-Jeghers syndrome


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Submitted
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Abstract

Mucocutaneous pigmentation is one of the features of the hereditary polyposis disorder Peutz-Jeghers syndrome (PJS). The diagnostic value of these pigmentation is unclear. Therefore, the presence of mucocutaneous pigmentation in PJS patients was compared to healthy subjects. Also, an attempt was made to determine any phenotype-genotype correlation between the expression of pigmentation in PJS patients and mutations in the PJS gene STK11. 34 Dutch PJS patients from 19 PJS families with proven characteristic hamartomatous intestinal polyposis, and 110 age and sex matched healthy controls were studied. Subjects were interviewed and examined for pigmentation by two observers and photographed in a standard fashion. 'PJS-like pigmentation' was defined as the distribution of dark melanin pigment spots typical for the syndrome. When available, past photographs were retrieved from hospital records for comparison. STK11 mutational analysis was used to determine the genotype in STK11 mutation positive PJS patients. PJS-like pigmentation was scored significantly more often in the patient group (71%) compared to the control group (15%) (p<0.001). The presence of 'PJS-pigmentation' had a sensitivity of 71%, a specificity of 86%, a positive predictive value of 60%, and a negative predictive value of 90% for the diagnosis Peutz-Jeghers syndrome in this study. In particular, pigmentation on the lips (p<0.001), buccal mucosa (p<0.01), eyelids (0.05), around the eyes (p<0.01), and around the nose (p<0.05) was significantly more common in PJS patients compared to controls. In the patients without PJS-like pigmentation, the skin and lip pigmentation had either disappeared with time (n=4), or had never been present at all (n=6). For the first time we report disappearance of buccal pigmentation in a PJS patient. Except for corresponding intense pigmentation in two families with separate missense mutations in codon 4 of the STK11 gene, no clear correlations between the pigmentation phenotype and STK11 genotype could be detected. Although characteristic, the diagnostic significance of PJS-like pigmentation is limited. In family studies, the diagnosis cannot be made or discarded on the basis of presence or absence of mucocutaneous pigmentations alone.

Introduction

When Peutz described the Peutz-Jeghers syndrome (PJS) in 1921 in a Dutch family, he was struck by the "most peculiar pigitations". He observed numerous small brown to dark bluish pigment spots located predominantly on the lips and the buccal mucous membranes as well as scattered around the mouth, the nose and the eyes of affected family members. They bore some resemblance to freckles, but were not affected in colour or size by sunlight. They had been present since early infancy and were clearly visible in the younger generation but had already disappeared in older family members. Together with intestinal polyposis Peutz believed that the presence of these pigmentation was pathognomonic for the syndrome.
Since the description by Peutz, this pigmented abnormality is considered as one of the hallmarks of this autosomal dominant syndrome, and the clinical diagnosis PJS is based on the triad of a positive family history, mucocutaneous pigmentation and hamartomatous polyposis of the gastrointestinal tract \(^1\),\(^2\). The presence of only one or two of these criteria in an individual, however, can make the clinical diagnosis difficult. With the identification of the PJS gene, \(STK11\) (also called \(LKB1\)), genetic testing became available to establish the diagnosis \(^3\)\(^\text{-}^6\). Still, melanin spots are an important clinical feature, as they may guide the clinician to the possibility of the diagnosis PJS in the individual patient. However, melanin spots occasionally are completely absent in PJS patients with hamartomatous polyps \(^7\). Conversely, although pigment spots present a very characteristic external feature in individuals carrying the PJS gene, they are not uncommon in persons without this syndrome. To what extent mucocutaneous pigmentation is of diagnostic significance is not clear. To determine the diagnostic value of mucocutaneous pigmentation in PJS, we carefully assessed the pigmentation pattern in 34 PJS patients from 19 different families, and in 110 healthy controls. Also, we investigated whether any phenotype-genotype correlations could be detected between the expression of mucocutaneous pigmentation and \(STK11\) mutations in PJS patients.

**Methods**

**Subjects**

PJS patients with proven characteristic hamartomatous intestinal polyposis were included in the PJS patient group. Possibly affected individuals from PJS families who reported a history of abdominal symptoms or pigmented changes, or both, but who had no documented intestinal polyps with the characteristic hamartoma histology, were excluded from this study. The control group consisted of healthy unrelated subjects with a negative personal and family history for PJS and other (hereditary) skin disorders. Informed consent was obtained from all participants. The study design was approved by the university medical ethics committee.

**Determination of pigmentation status**

All participants were interviewed about their medical history and pigmented changes and were examined by two observers (AMW and YKC). A scoring list was used including the age, sex, ethnic background, and the presence and quality of pigmentation on the lips, the mucosal membranes of the mouth, the nose, the area around the eyes, the eyelids, the dorsal and volar sided of the hands and the digits. In the PJS patient group photographs were taken in a standard fashion and compared with photographs taken in the past, if available. 'PJS-like pigmentation' was defined as the distribution of dark melanin pigment spots in a pattern typical for the syndrome, i.e. on the lips, the buccal mucosa, on the eyelids and/or around the nose and eyes.

**Mutation analysis**

Denaturing gradient gel electrophoresis (DGGE) and direct nucleotide sequencing was used to screen genomic DNA from the participating PJS patients for \(STK11\) mutations as described elsewhere \(^6\).
Chapter 12

Statistics

Differences were evaluated by the Chi-square test and the non-parametric Mann-Whitney-U test. Two-sided P values <0.05 were considered statistically significant.

Results

Thirty-four patients (17 men and 17 women) from 19 PJ S families, including members from the family originally described by Peutz 1, were included in the PJ S patient group. Ages ranged from 6 to 69 patients (median 36.5 years). The control group consisted of 110 healthy subjects (54 men and 56 women), aged 5 to 58 years (median 38.9 years). Patients and controls were investigated for the presence of melanin pigmentation at various sites, and for a "PJ S-like pigmentation pattern. Results are listed in Tables 1 and 2.

In PJ S patients, marked differences in presence, amount and intensity of pigmentation were observed within and between families. Melanin pigmentation occurred most consistently on the lips, i.e. in 27 patients or 79% of the cases. Pigmentation of the buccal mucosa, gums or palate was seen in 9 (27%) patients. The eyelids were involved in 12 (35%), the area around the eyes in 17 (50%), the area around the nose in 12 (35%), the palms of the hands in 6 (18%), and the (volar and interdigital spaces of the) digits in 12 (35%). In the control group, one or more pigment spots were found on the lips of 29 individuals (26%), on the buccal mucosa, gums or palate in 6 (6%), on the eyelids in 19 (17%), around the eyes in 23 (21%), around the nose in 16 (15%), on the palms of the hands in 37 (34%) and on the digits in 40 (36%). Pigmentation on the lips (p<0.001), buccal mucosa, gums or palate (p<0.01), on the eyelids (p<0.05), around the eyes (p<0.001) as well as around the nose (p<0.05) was significantly more common in PJ S patients compared to controls, whereas pigmentation on the palms of the hands and digits did not differ significantly between PJ S patients and controls.

Table 1. Presence of pigmentation by site in PJ S patients and controls. The presence of pigmentation at any site did not necessarily imply the classification PJ S-like pigmentation positive.

<table>
<thead>
<tr>
<th>Pigmentation site</th>
<th>PJ S patients (n=34)</th>
<th>Controls (n=110)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lips</td>
<td>27 (79%)</td>
<td>29 (26%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>9 (26%)</td>
<td>6 (5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eyelids</td>
<td>12 (35%)</td>
<td>19 (17%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Peri-oculocar skin</td>
<td>17 (50%)</td>
<td>23 (21%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peri-nasal skin</td>
<td>12 (35%)</td>
<td>16 (15%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Palms of hands</td>
<td>6 (18%)</td>
<td>37 (34%)</td>
<td>ns</td>
</tr>
<tr>
<td>Digits</td>
<td>12 (35%)</td>
<td>40 (36%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

In 24 of 34 (71%) PJ S patients a pigmentation pattern compatible with "PJ S-like pigmentation" was observed, whereas 16 of 110 (15%) controls would have been classified as PJ S based on their pigmentation alone (p<0.001). Thus, the presence of 'PJ S-like pigmentation' had a sensitivity of 71%, a specificity of 86%, a positive predictive value of 60%, and a negative predictive value of 90% for the diagnosis Peutz-Jeghers syndrome. In PJ S patients without "PJ S-
like pigmentation" (29%), these stigmata had already disappeared, or had never been present at all. The relationship between age and the presence of pigmentation in PJS patients is shown in Figure 1. Those who had lost pigmentation were older (median age 55 years, n=6) compared to the group in which pigmentation was present (median age 34 years, n=24) (p<0.0001). Four patients (12%) reported that they never had any pigmentation characteristic for PJS. Interestingly, in one PJS patient fading of lip pigmentation and complete disappearance of pigment spots on the buccal mucosa could be documented over a 21-year period (Figure 2).

### Phenotype-genotype correlation

STK11 mutations were found in DNA of 15 out of the 19 families. Participating PJS families and their mutations have been described before. In four families no mutation in the STK11 gene could be detected, suggesting either incomplete screening of the gene or the existence of a second, yet unidentified, PJS gene. As all mutations were different, no clear phenotype-genotype correlations could be detected in the PJS patient group. However, a missense mutation in exon 4 (G to A transversion at nucleotide 2528) was present in a family with 3 affected family members, all showing an extreme intensity of pigmentation. Interestingly, a second family with 4 affected family members showing similar intense pigmentation also revealed a missense mutation in exon 4: three associated substitutions at nucleotide 2432 (G to A), 2436 (G to A) and 2438 (C to A).

### Discussion

Distinctive melanin pigmentation is considered a hallmark of the PJS. These benign lesions appear of no clinical significance other than being a feature potentially guiding the physician to the diagnosis PJS. However, the sensitivity and specificity of this feature is not known, and a detailed study of pigmentation in PJS is lacking. The present study found PJS-like

<table>
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<tr>
<th>Pigmentation</th>
<th>PJS like</th>
<th>PJS patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Present</td>
<td>24 (71%)</td>
<td>16 (15%)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>4 (12%)</td>
<td>94 (86%)</td>
<td></td>
</tr>
<tr>
<td>Disappeared</td>
<td>6 (18%)</td>
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Table 2. Presence of PJS-like pigmentation in PJS patients and controls, p<0.001.
pigmentation in most PJS patients, but also in a subset of healthy individuals. The sensitivity and specificity of this sign for the diagnosis PJS was 71% and 86% respectively.

Figure 2: Fading of lip pigmentation and complete disappearance of buccal pigmentation in a male PJS patient, photographs taken at the age of 16 (A and C) and 37 (B and D) years, respectively.

Absence of pigmentation in some PJS patients may be (i) due to fading over time, or (ii) due to a lack of phenotypic expression of pigment spots in as reported by ~12% of patients in the present study. Gradual fading resulting in complete disappearance of pigment spots on the skin and lips is a well known phenomenon in PJS. However, pigment spots on the buccal mucosa and gingiva were thought to be permanent. For the first time, we report the disappearance of mucosal pigmentation in the mouth of a PJS patient in addition to fading of lip pigmentation (Figure 2). Furthermore, fading of pigmentation in PJS seems related to age; patients who had lost pigmentation were older than those in whom melanin pigmentation was present (Figure 1). Melanin spots in PJS are not present at birth, appear during early infancy or childhood and gradually increase in number and intensity reaching a maximum at puberty. In subsequent years, pigment spots may slowly fade and sometimes disappear. Interestingly, marked inter-family and intra-family variation in the expression of pigmentation exist. An attempt was made to compare the pigmentation phenotype in PJS families to the genetic defect. Two families with corresponding intense pigmentation had separate missense mutations in codon 4 of the STK11 gene. Large genotype-phenotype studies are required to further investigate such a correlation.

Strikingly, 15% of healthy controls had a PJS-like pigmentation pattern. Two forms of physiological pigmentation exist: ephelides and lentigines. PJS-pigment spots show the greatest resemblance to lentigines, which are darkly colored and not influenced by light, develop during childhood or early adult life, and can persist for years. In contrast, ephelides (or freckles) are light brown colored, induced by light and fading with decreased light-exposure. PJS-like pigmentation can also be mimicked by other disorders. Laugier-Hunziker syndrome is a rare pigmented abnormality of the lips and oral mucosa, also affecting the nails. In contrast to PJS, it is an acquired disorder appearing later in life, twice as common in females as in males. A rare hereditary pigmented disorder with a similar age of onset as PJS is the multiple lentigines or LEOPARD syndrome. This autosomal dominant disorder shows cutaneous lesions.

112
corresponding to PJS, however, lentigines in LEOPARD syndrome are solely present on the skin and not on mucosal surfaces. The LEOPARD syndrome comprises lentigines, ECG abnormalities, ocular hypertelorism, obstructive cardiomyopathy, pulmonary valve stenosis, abnormalities of the genitalia, retardation of growth, and deafness, but is not associated with gastrointestinal polyposis. In addition to physiological pigmentation, these rare conditions should be considered in the differential diagnosis of PJS-like pigmentation.

To date, the pathogenesis of PJS-like pigmentation is not understood. Histologically, PJS pigment spots show accumulation of melanin in cells of the basal layer of the epidermis with an apparently normal number of size of melanocytes, which is not truly distinctive from physiological forms of pigmentation. As with other inherited patterned pigment disorders, aberration of the embryological development of the pigment pattern could be the underlying mechanism. Melanocytes of the skin and mucous membranes migrate from the neural crest to the embryonic epidermis during the first three months of gestation. Those neural crest cells may be the target of mutant genes in inherited pigment pattern disorders. Abnormal pigmentation in PJS becomes manifest during postnatal life. Several PJS patients in our study mentioned a rapid increase of the number of pigment spots during puberty, possibly reflecting a (sex-) hormonal effect. This would also be consistent with the gradual disappearance of pigment spots in later life. Interestingly, Perusse et al. described the induction of PJS-like pigmentation on the lips and oral cavity by estrogen-replacement therapy in a fifty-year-old female without manifestations of PJS. Discontinuation of estrogen resulted in regression of oral pigmentation. Estrogen derivatives can increase the production of beta-melanocyte stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH). Both hormones induce melanin production in melanocytes, providing an explanation for a possible effect of estrogen on pigmentation. The appearance of PJS-like pigment spots on the lips and oral mucosa has also been described in the course of chemotherapy for ovarian cancer, and in association with malignancy in patients without a history of PJS. Recently, Boardman et al. investigated patients with PJS-like pigmentation but no polyposis, which they designated as isolated mucocutaneous melanotic pigmentation (IMMP). Females with IMMP had an increased risk for breast and gynecologic cancer, further supporting an hormonal effect involved in the development of pigmentary abnormalities.

In conclusion, the present study shows that PJS-like pigmentation can be found in most PJS patients. Although mucocutaneous pigment spots at distinctive sites can be a clue to the presence of PJS, it is not pathognomonic, nor does its absence exclude PJS. The specificity is limited, since ~15% of healthy controls had "PJS-like pigmentation". Age-related expression, and inter-family and intra-family differences in expression of pigmentation deserve consideration in the clinical evaluation of suspected PJS patients and at-risk individuals of known PJS families.

References