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Chapter 11

Nasal polyposis in PJS: a distinct entity with pre-malignant potential?


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Chapter 11

Nasal polyposis in Peutz-Jeghers syndrome: a distinct entity with pre-malignant potential?

Abstract:

Peutz-Jeghers syndrome (PJS) is an autosomal dominant hamartomatous polyposis syndrome of the gastrointestinal tract, caused by a germline STK11/LKB1 mutation. In the original report by Peutz, nasal polyposis was described. Recently, we reported a molecular-genetic association between nasal polyposis and PJS. The present investigation further explores the occurrence and pathogenesis of PJS related nasal polyposis. 51 PJS patients from 18 PJS families, 120 family members and a sample of 3289 random individuals were questioned for the presence of nasal polyposis. In addition, 12 PJS related nasal polyps and 28 sporadic nasal polyps were analysed for loss of heterozygosity (LOH) at 19p13.3 (STK11/LKB1 locus), eosinophilia, squamous metaplasia, dysplasia, and immunohistochemical expression of COX-2 and p53. Also, a PJS related carcinoma of the nasal cavity was investigated for inactivation of STK11/LKB1. Nasal polyposis occurred in 8 of 51 (17%) PJS patients, none of non-affected family members (p<0.001) and 223 of 3289 (6.8%) individuals in the general population (p<0.05). LOH was found in 4 of 8 PJS related nasal polyps, and in none of the sporadic nasal polyps (p=0.002). A carcinoma of the nasal cavity of a PJS patient with nasal polyposis did not show LOH; also no somatic mutation in STK11/LKB1 nor CpG-island hypermethylation of the promoter-region was found. Peutz-Jeghers syndrome related nasal polyps showed less eosinophilia than sporadic nasal polyps (p<0.001). COX-2 expression was found in 11 of 12 PJS related nasal polyps and 19 of 28 sporadic nasal polyps (p>0.05). Nuclear overexpression of p53 was not found in any polyp. Nasal polyposis occurs in a significant number of Dutch PJS patients; one of whom developed a carcinoma in the nasal cavity. The finding of LOH at the STK11/LKB1 locus, and the absence of eosinophilia suggests a distinct pathogenesis compared to sporadic nasal polyposis.

Introduction

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant disorder, characterized by hamartomatous polyposis of the gastrointestinal tract and melanin pigmentation of the skin and mucous membranes. PJS patients have an increased risk for developing cancer at relatively young age. Malignancies occur in the gastrointestinal tract, and also in a variety of extra-gastrointestinal sites. PJS is caused by a germline mutation in the STK11/LKB1 tumour suppressor gene on chromosome 19p13.3. Molecular analysis of hamartomas and carcinomas from PJS patients has shown loss of heterozygosity (LOH) at chromosome 19p13.3, indicating inactivation of the wild type STK11/LKB1 gene.

In 1921, the Dutch physician Peutz described the first PJS family with both nasal and gastrointestinal polyposis. Evaluation of this original family revealed that 6 of 22 patients suffered from nasal polyposis, and one of these individuals developed a nasopharyngeal carcinoma. Nasal polyposis associated with PJS has also been described by others, and
recently we reported loss of the wild type \textit{STK11/LKB1} allele in PJS related nasal polyps, providing molecular genetic support for the association between nasal polyposis and PJS \cite{19}.

In the general population, nasal polyposis is a relatively common disorder \cite{20}. This condition is thought to be an allergic inflammatory disorder, with common pathogenesis to asthma \cite{21}. The majority of nasal polyps show extensive infiltration of eosinophilic leukocytes, consistent with a hypersensitivity reaction \cite{22}. Squamous metaplasia is found in a small subset of lesions. Nasal polyps are not considered a pre-neoplastic entity, with reports about malignant degeneration lacking, and oncogenic mutations not described to our knowledge. Case reports of nasal polyposis associated with PJS \cite{13-18}, finding LOH in nasal polyps of PJS patients \cite{19}, and the co-occurrence of nasal polyposis and a carcinoma of the nasal cavity in a PJS patient \cite{12}, suggests that these nasal polyps follow a distinct pathogenesis with neoplastic potential.

The present study further investigates the relationship between nasal polyposis and PJS. The presence of nasal polyposis in well-documented Dutch PJS-families was assessed. PJS related and sporadic nasal polyps were studied for LOH at 19p13.3, the presence of eosinophilia, squamous metaplasia and dysplasia. Also, the expression of COX-2 and p53 was evaluated. A carcinoma of the nasal cavity from a PJS patient with nasal polyposis was investigated for inactivation of \textit{STK11/LKB1}.

\section*{Methods}

\textit{Patients and tissue specimens:}

Fifty-one PJS patients, from 18 well-documented PJS-families \cite{11, 12, 23, 24} were included. The clinical diagnosis (gastrointestinal polyposis and mucocutaneous pigmentation) was confirmed by histopathologic review of gastrointestinal hamartomas by an experienced pathologist (GJAO). In all but one family germline \textit{STK11/LKB1} mutations were identified. From the above study group, 35 PJS patients were questioned about a medical history of nasal polyposis. In addition, data from 16 deceased but well documented PJS patients from the original family described by Peutz were used. Clinical information about these patients was obtained from medical charts, previous publications \cite{1, 12, 25}, and interviews with first-degree relatives. As controls, 84 non-affected (genetically related) family members, and 36 spouses were questioned about the occurrence of nasal polyposis. The protocol was approved by the medical ethics committee of the University Hospital Rotterdam, Erasmus University. Informed consent was given by all participating individuals. Also, 3268 individuals were questioned about the presence of physician-diagnosed nasal polyposis, using a random sample of the general population frequently contacted for market research purposes.

From 4 PJS patients with nasal polyposis, 12 nasal polyps and one carcinoma of the nasal cavity were available for study. The specimens were collected from the pathology departments of several hospitals in The Netherlands. A control group consisted of 28 sporadic nasal polyps from age and gender matched patients without PJS, cystic fibrosis, Kartagener syndrome or aspirin hypersensitivity. The sporadic polyps were removed between 1996 and 1998 at the Academic Medical Center in Amsterdam, The Netherlands, and were selected randomly from the pathology archives.
Table 1: Germline STK11/LKB1 mutation, number of Peutz-Jeghers syndrome (PJS) patients, and number of PJS patients with nasal polyposis in PJS families with nasal polyposis.

<table>
<thead>
<tr>
<th>Family</th>
<th>STK11 exon/intron; codon</th>
<th>nucleotide change</th>
<th>alteration mRNA</th>
<th>Number of PJS patients</th>
<th>PJS patients with nasal polyposis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Deletion exon 2-3; codon 98-150 loss of 57 codons deletion of 57 amino acids</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Missense exon 7; codon 297 4016 G/A AGG/AAG</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Insertion exon 1; codon 66 535 ins T frameshift 162 TGA/stop</td>
<td>22</td>
<td>6²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Not previously published mutation.
² Including patient IV.12 (reference 12) who developed a carcinoma of the nasal cavity.

Tissue preparation and DNA isolation:

Formalin-fixed and paraffin-embedded samples were cut into 5 µm sections, mounted onto glass slides, and H&E stained for histopathologic examination. Polyp and carcinoma epithelium was carefully microdissected from hematoxylin stained slides. For control wild type DNA, DNA isolated from stromal tissue from the same sample was used. The microdissected tissue was collected in microcentrifuge tubes containing 10-15 µl proteinase K solution (50 mM Tris-HCl pH 8.0, 0.2% Tween-20 and 100mg/ml proteinase K), and incubated overnight at 56 °C. Samples were heated to 96 °C for 10 minutes to inactivate the proteinase K.

LOH at 19p13.3; mutation analysis; CpG-island hypermethylation:

Analysis of loss of heterozygosity (LOH) was done as described previously, comparing DNA from microdissected (epithelial) polyp or carcinoma tissue with normal tissue from the same patient. The polymorphic microsatellite markers D19S886 and D19S565, flanking the STK11/LKB1 gene on chromosome 19p13.3, were used (for primer sequences see www.gdb.org). If a marker was homozygous in normal DNA, polyps of this patient were defined as non-informative for the tested marker. DNA from a PJS related carcinoma of the nasal cavity was also studied for somatic mutations in STK11 by exon-sequencing using previously reported primers, and for CpG-island methylation induced silencing of the STK11/LKB1 promoter region using a methylation-specific PCR (MSP) as described.

Histopathologic examination:

H&E stained slides of nasal polyps were examined for the presence of eosinophils, squamous metaplasia, and dysplasia by two observers (JJK and GJAO) in a coded fashion. The presence of eosinophils in the stroma underneath the surface epithelium was assessed in a semiquantitative manner (0 = none/only a few eosinophils; + = moderate number of eosinophils; ++ = large number of eosinophils). Squamous metaplasia and dysplasia were assessed as present or absent.
Immunohistochemistry for COX-2 and p53:

Immunohistochemistry was performed on 5 \( \mu \)m sections of formalin-fixed, paraffin-embedded samples as described previously. Primary monoclonal antibodies used were: antibody #160112 against COX-2 (Cayman Chemical Co., Ann Arbor, MI, USA) at a dilution of 1:100; and DO7 (Dako, Glostrup, Denmark) against p53 at a dilution of 1:200. For antigen retrieval, slides were boiled for 10 min. in citrate buffer. Slides were incubated with the primary antibody overnight at 4 °C (COX-2) or for 1 h at room temperature (p53). As positive control, a known positive colorectal carcinoma was used. Immunostained slides were scored by two observers (JK and GJAO) in a coded fashion. Epithelial COX-2 staining was assessed semiquantitatively on a scale from 0-+++ (0: no staining; +: weak staining; ++: moderate staining; +++: intense staining), considering the staining-intensity and % of positive cells. p53 staining was considered positive if >10% of nuclei were positive. Nuclear p53 staining restricted to the basal (proliferative) compartment of squamous metaplasia was not considered positive.

Statistics

Comparisons between groups were made using the Mann-Whitney test for the presence of eosinophils and COX-2 expression, and the Fisher's exact test for the presence of LOH, squamous metaplasia, and dysplasia. A p-value < 0.05 was considered statistically significant. All p-values were two-sided.

Results

Nasal polyposis in Peutz-Jeghers syndrome patients:

Nasal polyposis occurred in 8 out of 51 PJS patients (16%), whereas none of the non-affected family members or spouses of PJS patients reported nasal polyposis (p<0.001). For comparison, in the general population, 223 out of 3268 (6.8%) random individuals reported physician-diagnosed nasal polyposis at any time during their life (p=0.05).

The 8 PJS patients with nasal polyposis were from 3 different families with established germline STK11/LKB1 mutations (Table 1). Nasal polyposis did not occur in PJS patients from 15 other families. Clustering of nasal polyposis and PJS was found in one family. In this well documented family, 6 out of 22 (27%) PJS patients had nasal polyposis. Detailed clinical information was available from 4 PJS patients with nasal polyposis, from 3 different PJS families. Three of these patients suffered from recurrent and serious nasal polyposis, for which multiple polypectomies were performed. One PJS patient with nasal polyposis had a pulmonary adenoma at age 38, and also developed a moderately differentiated carcinoma of the nasal cavity at age 52 (patient IV.12, ref 12). Figure 1 is a picture of this patient at the age of 24 years, at which time she already suffered from nasal polyposis.

Inactivation of STK11:

Twelve nasal polyps from 4 PJS patients and 28 sporadic nasal polyps were studied for LOH of two polymorphic markers flanking the STK11/LKB1 locus 19p13.3. LOH was found in 4 of 8 informative nasal polyps from 2 PJS patients from different families. DNA from two other
polyps from different patients did not amplify consistently. Both markers were non-informative in DNA of one patient, from whom 2 nasal polyps and a carcinoma of the nasal cavity were selected for study. LOH was not found in 23 informative sporadic nasal polyps from the controls. The difference in frequency of LOH in PJS-related nasal polyps compared to sporadic nasal polyps was statistically significant (p=0.002) (Table 2).

DNA isolated from a carcinoma of the nasal cavity from a PJS patient with nasal polyposis was studied for inactivation of \textit{STK11/LKB1}. Markers used for LOH analysis were non-informative (see above). However, sequencing revealed that tumor DNA contained wild type \textit{STK11/LKB1} and an allele with the \textit{STK11/LKB1} germline mutation (insert T at codon 66, exon 1), indicating the absence of LOH at 19p13.3. Also, no somatic mutation in \textit{STK11/LKB1} or CpG-island hypermethylation within the promoter region of \textit{STK11/LKB1} was found.

**Histological comparison of PJS and sporadic nasal polyps:**

Twelve nasal polyps from 4 PJS patients were compared to 28 sporadic nasal polyps from controls. There were significantly less eosinophils in nasal polyps of PJS patients compared to the sporadic nasal polyps (p<0.001) (Table 2 and Figure 2). Only 1 of 12 nasal polyps from PJS patients demonstrated a moderate number of eosinophils, whereas 23 of 28 sporadic nasal polyps showed moderate (n=8) or large (n=15) numbers of eosinophils (Table 2, Figure 2, 3a and 3b). Squamous metaplasia was present in one poly of a PJS-patient (8%), and in 6 sporadic nasal polyps (21%) (p=0.65) (Table 2). No dysplasia was found in any of the polyps.

![Figure 1. PJS patient (family III) with nasal polyposis at age of 24. The perinasal asymmetry and the broadened nasal bridge were attributed to nasal polyposis by Dr. Th. van Wijk, who published this picture in his thesis in 1955. The patient died from a carcinoma of the nasal cavity (Figure 2D) at the age of 52.](image1)

![Figure 2. Distribution of eosinophilia in nasal polyps from PJS patients and sporadic nasal polyps, assessed in a semiquantitative manner as absent/weak, moderate or strong. Eosinophilia was significantly more common in sporadic than PJS related nasal polyposis (p<0.001).](image2)
Immunohistochemistry for COX-2 and p53:

PJS related and sporadic nasal polyps were evaluated for the immunohistochemical expression of COX-2 and p53. COX-2 expression was studied since it was recently found to be up-regulated in PJS related hamartomatous polyps; p53 staining was assessed because overexpression was reported in 96% of nasopharyngeal carcinomas and 79% of adjacent dysplastic lesions, suggesting a potential as marker for (pre-)malignant growth of nasopharyngeal epithelium. COX-2 expression (ranging from + to ++++) could be found in the cytoplasm of epithelial cells of 11 out of 12 nasal polyps of PJS patients, and 20 out of 28 sporadic nasal polyps (Figure 2c). The degree of COX-2 expression was assessed semiquantitatively on a scale from 0 (negative) to +++ (strong COX-2 expression). PJS related nasal polyps (median ++; range 0-++++) had more COX-2 expression than sporadic nasal polyps (median +; range 0-+++), although the difference was not significant (p=0.10). COX-2 expression did not correlate with LOH in PJS related polyps. COX-2 expression was also present in a PJS-related carcinoma of the nasal cavity (Figure 2). Nuclear over-expression of p53 (>10% positive nuclei) was not found in PJS related or sporadic nasal polyps.

Discussion

The present study further investigates the association between nasal polyposis and Peutz-Jeghers syndrome, first reported by Peutz in 1921. Although a minority of PJS patients suffer from nasal polyposis, it may be of significance. One PJS patient with nasal polyposis died from a carcinoma of the nasal cavity. Furthermore, inactivation of the STK11/LKB1 tumor suppressor gene by LOH was found in PJS related nasal polyps, indicating a neoplastic clonal nature of these lesions.

Our study suggests a different pathogenesis for PJS-related compared to sporadic nasal polyps. LOH at the STK11/LKB1 locus 19p13.3 can be found in PJS related polyps but not in sporadic lesions. To date, no report of genetic alterations in sporadic nasal polyps exists. In general, nasal polyps are characterised by extensive infiltration of eosinophilic inflammatory cells, consistent with an allergic/inflammatory pathogenesis. Nasal polyps from PJS patients...
had significantly fewer eosinophils than sporadic nasal polyps (p<0.001). Similar observations are reported in nasal polyps associated with other specific syndromes. For example, those from patients with cystic fibrosis or Kartagener's syndrome also lack extensive eosinophilia\textsuperscript{22, 30}.

Only 16\% of the PJS patients in our study had nasal polyposis, found in 3 of 18 PJS families studied. In the family originally described by Peutz, 6 out of 22 PJS patients had nasal polyposis, but not their 54 non-affected family members and spouses, making a PJS-independent cause unlikely. Familial clustering may occur and large genotype-phenotype investigations are necessary to elucidate whether nasal polyposis is correlated with a specific genotype, which could aid in the development of surveillance strategies. The relatively low incidence of nasal polyposis in PJS presumably explains the limited number of reports of this association. Of note, nasal polyposis is common in the general population\textsuperscript{21}. Using a questionnaire in a random sample of Dutch citizens, the prevalence of physician-diagnosed nasal polyps was 6.8\%. Thus, nasal polyposis can not serve as a marker for PJS, and nasal polyps of PJS patients can not exclusively be attributed to the syndrome.

<table>
<thead>
<tr>
<th>LOH 19p</th>
<th>Eosinophilia\textsuperscript{1}</th>
<th>Squamous metaplasia</th>
<th>COX-2\textsuperscript{2}</th>
<th>P53 \textsuperscript{p}</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;0.01</td>
<td>P&lt;0.001</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Peutz-Jegher polyops</td>
<td>4/8</td>
<td>1/12</td>
<td>1/12</td>
<td>11/12</td>
</tr>
<tr>
<td>Sporadic polyops</td>
<td>0/23</td>
<td>23/28</td>
<td>6/28</td>
<td>20/28</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Moderate or strong infiltration of eosinophils
\textsuperscript{2} Weak (+), moderate (+++) or strong (+++) expression
\textsuperscript{3} Calculated using semiquantitative score from 0-+++}

One patient with PJS, nasal polyposis and carcinoma of the nasal cavity was identified. Genetic analysis of this carcinoma could not prove inactivation of \textit{STK11/LKB1} by LOH, somatic mutation, or CpG-island hypermethylation in the promoter region of \textit{STK11/LKB1}. However, this does not exclude an association between PJS, \textit{STK11/LKB1} and the development of a carcinoma of the nasal cavity in this patient. Inactivation may have occurred by an intronic mutation resulting in alternative splicing, or by large deletions not detected by exon sequencing of short strands of DNA isolated from paraffin-embedded tissue. Alternatively, haploinsufficiency may suffice to explain \textit{STK11/LKB1} mediated tumor-growth, as suggested by the analysis of tumors from heterozygous \textit{Stk11/Lkb1} knockout mice\textsuperscript{28, 31}. The co-occurrence of nasal polyposis and a carcinoma of the nasal cavity in a PJS patients suggests the pre-malignant potential of PJS related nasal polyps. The finding that nasal polyps and a carcinoma of the nasal cavity from PJS patients over-express COX-2 may provide a rationale for experimental chemopreventive treatment with NSAIDs or COX-2 inhibitors in PJS patients with (severe) nasal polyposis.

In conclusion, nasal polyposis associated to PJS should be considered as extra-intestinal hamartomas. The absence of eosinophils compared to sporadic nasal polyps indicates a distinct pathogenesis, in which LOH at the \textit{STK11/LKB1} locus 19p13.3 seems involved. The potential
neoplastic risk of nasal polyps in PJS should be considered in the clinical management of these patients.

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References


