Etiology and outcome of PFAPA (Periodic Fever, Aphthous Stomatitis, Pharyngitis and Adenitis) syndrome among patients operated with tonsillectomy in childhood

Ulla Lantto
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ETIOLOGY AND OUTCOME OF PFAPA (PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS AND ADENITIS) SYNDROME AMONG PATIENTS OPERATED WITH TONSILECTOMY IN CHILDHOOD

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Abstract

Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a syndrome characterized by regular, high-fever episodes with healthy periods in between. In a classic phenotype of the syndrome, the fevers begin in childhood before the age of five, and fever flares are accompanied by aphthous stomatitis, pharyngitis, and/or cervical adenitis. The etiology of the syndrome is unknown, but tonsillectomy (TE) has been shown to be an effective treatment for the disease.

The purposes of this study were as follows: (1) to assess the long-term outcome of PFAPA patients treated by TE with either the classic or incomplete phenotype (later onset of the disease and/or missing oropharyngeal symptoms), (2) to compare the health and growth of PFAPA patients with healthy controls, and (3) to compare the histological and microbiological findings of the tonsils of PFAPA patients with controls via conventional and modern sequencing technologies.

In this approximately 9-year follow up, 97% (n = 56) of patients with the classic phenotype and all patients (n = 50) with the incomplete phenotype achieved a prompt and constant response after TE. There were no differences in either the length of fever episodes or flares between patients with both the classic and incomplete phenotypes.

The health and growth of 119 PFAPA patients was compared to that of sex- and age-matched controls (n = 230), and no differences in prevalence of chronic diseases or growth were found between the groups. Infections, oral thrush, and pollen allergy were more common in the history of the PFAPA patients than in the controls.

Microbiological and histological findings of the tonsils of PFAPA patients (n = 31) were compared with the findings of the controls (n = 24) who had undergone TE for other reasons. Biofilm formation and Candida albicans were more frequently found among PFAPA patients than the controls, but Staphylococcus aureus, varicella zoster, and herpes simplex viruses were more common in the controls. While comparing the bacterial microbiota between the groups, we found significant differences in the presence and relative abundance of many bacteria. For example, Cyanobacteria were more common and abundant in the case samples than in the controls.

Because the long-term outcome after TE was excellent, both in classic and incomplete PFAPA patients; a new diagnostic criteria for the syndrome is proposed. The microbes of the tonsils in PFAPA patients differ from that of the controls, which may play an important role in triggering the inflammatory processes that lead to symptoms of PFAPA.

Keywords: biofilm; children; growth; health; microbes; outcome; periodic fever; PFAPA, periodic fever, aphthous stomatitis, pharyngitis and adenitis; tonsillectomy
Lantto, Ulla, Lapsena nielurisaleikkuksella hoidetun PFAPA (periodic fever, aphthous stomatitis, pharyngitis ja adenitis) oireyhtymän ennuste ja etiologiset tekijät.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala

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**Tiivistelmä**

Periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome, on oireyhtymä, jossa potilaat kärsivät hyvin säännöllisesti ilmaantuvista, toistuvista kuumejaksoista, toistuvista kuumejaksoista, joiden välillä potilaat ovat terveitä. Klassisessa tautimuodossa kuumeilut alkavat lapsuudessa ennen viidennän vuoden ikää ja kuumevaiheeseen liittyvät liitännäisoireita: suun limakalvojen rakkuloita, nelutulehdusta ja/tai kaulan imusolmukkeiden suurentumista. Oireyhtymän syytä ei tiedetä, mutta nielurisaleikkaus (TE) on osoittautunut tehokkaaksi hoidoksi.

Tutkimuksen tarkoituksena oli (1) arvioida PFAPA potilaiden vointia pitkäaikaisseurannassa TE:n jälkeen ja vertailla taudin- ja nielurisoihin niiden PFAPA potilaiden välillä, joilla oli klassinen PFAPA tai epätyypillinen PFAPA. (2) Lisäksi tutkimme myös TE:llä hoidettujen PFAPA potilaiden sairastuvuutta, yleistä terveydentila ja kasvua vertaamalla näitä sukupuoli- ja ikävakioituihin kontrolliin ja (3) selvitimme mikrobiologisia ja histologisia löydöksiä PFAPA potilaiden nielurisoiissa verrattuna muista syistä TE:ssa käyneiden lasten nielurisoihin.

Tässä noin yhdeksän vuoden seurannassa TE:n jälkeen oli täysin parantunut 97% (n = 56) potilaista, joilla oli klassinen PFAPA, ja kaikki (n = 50) potilaat, joilla oli epätyypillinen PFAPA (tauti oli alkanut viiden ikävuoden jälkeen ja/tai klassiset liitännäisoireet puuttuivat). Kuumeprofilait äivät muilta osin erooneet ennen nielurisaleikkausta näissä ryhmissä.

PFAPA potilaaiden (n = 119) kasvu ja yleinen terveydentila eivät erooneet väestökontrolleista (n = 230). Krooniset ja autoimmuunisairaudet olivat yhtä harvinaisia molemmassa ryhmässä. Potilaat raportoivat sairastaneensa enemmän infektioida ja samasta lapsuudessa ja heillä oli enemmän siitepölyallergioita.


**Asiasanat:** biofilm; ennuste; kasvu; lapset; mikrobit; nielurisaleikkaus; PFAPA, periodic fever, aphthous stomatitis, pharyngitis and adenitis; terveys; toistuva kuume
To My Family
Acknowledgements

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Ulla Lantto
### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAPS</td>
<td>Cryopyrin-associated periodic syndrome</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>FMF</td>
<td>Familiar Mediterranean fever</td>
</tr>
<tr>
<td>HIDS</td>
<td>Hyperimmunoglobulinemia D</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IL</td>
<td>Interleucin</td>
</tr>
<tr>
<td>INF</td>
<td>Interferon</td>
</tr>
<tr>
<td>KELA</td>
<td>Social Insurance Institution of Finland</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>OTU</td>
<td>Operational taxonomic unit</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SAA</td>
<td>Serum amyloid A</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SND</td>
<td>Standard normal deviate test</td>
</tr>
<tr>
<td>TE</td>
<td>Tonsillectomy</td>
</tr>
<tr>
<td>TEA</td>
<td>Adenotonsillectomy</td>
</tr>
<tr>
<td>TRAPS</td>
<td>Tumor necrosis factor receptor-associated periodic syndrome</td>
</tr>
</tbody>
</table>
List of original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


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1 Introduction

In 1987, Marshall published a series of 12 patients with earlier unknown periodic fever syndrome. The most remarkable feature was a clockwork periodicity of fevers with healthy periods in between. The onset of the syndrome mainly occurred before the age of five years, and the most common symptoms at the time of fevers were stomatitis, pharyngitis, and cervical adenopathy, but other clinical symptoms were also seen. The duration of fever flares was on average five days, after which the symptoms settled down spontaneously but reoccurred in stereotypical cycles after every 2–9 weeks. The etiology of the syndrome was unknown (Marshall et al., 1987).

The term Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis, PFAPA, was introduced in 1989, (Marshall et al., 1989) and the first follow-up of 83 PFAPA patients was published 10 years later. The syndrome seemed most often to last for many years. While the etiology of the syndrome was not known, diagnostic criteria were stated due to its cardinal symptoms described by Marshall et al., (Marshall et al., 1987; Thomas et al., 1999). Antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) were ineffective. Fever flares reacted strongly to one dose of systemic corticosteroids, but that could not prevent upcoming episodes. (Thomas et al., 1999) Instead, tonsillectomy (TE) or adenotonsillectomy (TEA) operations for four PFAPA patients gave promising results (Abramson et al., 1989). Since then, the strong effectiveness of TE/TEA has been proven in randomized trials (Burton et al., 2014; Garavello et al., 2009; Renko et al., 2007).

The cytokine and immunological pattern of the disease, the multiple-year duration, and the good response to corticosteroids suggest that PFAPA is an autoinflammatory disease (Dytrych et al., 2015; Stojanov et al., 2011; Wekell et al., 2016). Unlike classic autoinflammatory diseases, PFAPA syndrome has spontaneous healing potential. The long-term prognosis of PFAPA syndrome is thought to be generally good, and its most effective treatment is TE, which refers more to infectious than autoinflammatory etiology (Garavello et al., 2009; Renko et al., 2007; Wekell et al., 2016; Wurster et al., 2011b). The tonsils have a key role in PFAPA and one theory suggests lymphocytes driven by microbes or other environmental stimuli might trigger autoinflammation in the syndrome (Dytrych et al., 2015; Stojanov et al., 2011). Still, the etiology of the PFAPA syndrome remains unknown. Because the diagnosis is based on a combination of symptoms, we may see only part of the variation of clinical pictures of this syndrome.
To understand the mechanisms behind PFAPA syndrome, in this study the long-term outcome and co-morbidities of the disease were investigated after the operative treatment as well as the histology and microbes of the tonsils compared to controls.
2 Review of the literature

2.1 Definition of PFAPA syndrome

In 1987, the first series of 12 patients with periodic fever syndrome was published and two years after the first diagnostic frames with the term periodic fever, aphthous stomatitis, pharyngitis and adenitis, PFAPA, were stated (Marshall et al., 1987; Marshall et al., 1989). Due to the main characteristics of those 12 patients Thomas et al., described the first diagnostic criteria for PFAPA syndrome in 1999. The patient has to have regularly recurring fever flares with an early age at onset (< 5 years) with constitutional symptoms in the absence of upper respiratory infection with at least one of the following clinical signs during the fever flares: aphthous stomatitis, cervical lymphadenitis, or pharyngitis. Cyclic neutropenia must be excluded. The patient must be completely asymptomatic between fever episodes and the growth and development normal (Thomas et al., 1999) (Table 1).

The Pediatric Rheumatology International Trials Organization (PRINTO) has also defined diagnostic criteria of PFAPA syndrome due to the data of Euro-fever register, which accompany the Thomas criteria, but has not been published (www.printo.it).

Table 1. The diagnostic criteria of PFAPA syndrome according to Thomas et al. (1999).

<table>
<thead>
<tr>
<th>Thomas diagnostic criteria of PFAPA syndrome</th>
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<tbody>
<tr>
<td>Regularly recurring fevers with an early age of onset (&lt; 5 years of age)</td>
</tr>
<tr>
<td>Constitutional symptoms in the absence of upper respiratory infection with at least 1 of the following clinical signs: Aphthas, Adenitis, Pharyngitis</td>
</tr>
<tr>
<td>Exclusion of cyclic neutropenia</td>
</tr>
<tr>
<td>Completely asymptomatic interval between episodes</td>
</tr>
<tr>
<td>Normal growth and development</td>
</tr>
</tbody>
</table>

As the etiology of the syndrome has been unknown, the diagnosis of PFAPA syndrome is based on typical symptoms. Since the awareness of PFAPA has increased, the syndrome has shown to have a much wider variety of symptoms than Thomas’ criteria predicated, and therefore the practice of diagnostics varies in different centers (Hofer et al., 2014).

The age criterion has been questioned, because the syndrome has been reported to begin also after the age of five or even among the adults (Hofer et al., 2014; Marshall et al., 1987; Padeh et al., 2008; Vitale et al., 2016) and in some centers
aphthous stomatitis, pharyngitis, and adenitis are not required for the diagnosis (Licameli et al., 2008; Licameli et al., 2012; Manthiram et al., 2017b; Renko et al., 2007). The most distinctive features of the syndrome seem to be the clockwork periodic fevers with the alternation of stereotypical fever episodes and healthy periods (Gattorno et al., 2009; Long, 1999; Vanoni et al., 2018).

2.2 Symptomology and natural course of the disease

PFAPA patients suffer from periodic fevers reoccurring with a similar pattern after every 2–5 weeks. The fever flare lasts for 2–7 days, and the body temperature rises typically over 39°C. C-reactive protein (CRP) levels and leucocyte accounts increase in most patients. Throat cultures for Streptococcus pyogenes are negative. The flare ends spontaneously. Between fever flares, the patients are completely healthy (Feder & Salazar, 2010; Tasher et al., 2006; Thomas et al., 1999; Wurster et al., 2011b). The symptoms of PFAPA syndrome begin most often from the age of 11 months to 4 years (Feder & Salazar, 2010; Førsvoll et al., 2013; Tasher et al., 2006; Wurster et al., 2011b), but the syndrome has been described to begin also at older age, even among adults (Cantarini et al., 2017; Hofer et al., 2014; Padeh et al., 2008; Vitale et al., 2016). At the time of PFAPA fever flares, family members and other close contacts of the patient remain well (Feder & Salazar, 2010).

During the fever flares, PFAPA patients are very ill. According to a European multicenter PFAPA cohort study of 301 cases, most patients have aphthous stomatitis, cervical lymphadenitis, pharyngitis, or exudative tonsillitis at the time of a fever, and over 30% patients have additional symptoms like abdominal pain, nausea, diarrhea, and arthralgias (Federici et al., 2015; Hofer et al., 2014). Occasionally, patients may skip an episode of a fever and then return to periodic fevering (Feder & Salazar, 2010), but most still have on average 10–11 episodes per year (Thomas et al., 1999). The growth and development are supposed to be normal, as stated in classic diagnostic criteria, nonetheless comparisons to healthy controls have not been made (Marshall et al., 1987; Thomas et al., 1999).

PFAPA syndrome can heal spontaneously, but it tends to be long-lasting. The mean duration of the syndrome is 3–6 years (Feder & Salazar, 2010; Førsvoll et al., 2013; Wurster et al., 2011b), but reports of ongoing symptoms after 24 years of follow up also exist (Wurster et al., 2011b). According to Feder and Salazar (2010), 20% of 105 PFAPA patients healed spontaneously in a 10-year follow up, and spontaneous healing was seen also in a Finnish randomized study comparing TE versus six months of follow up. Half of the 12 PFAPA patients of the follow up -
group healed spontaneously. The duration of PFAPA symptoms before inclusion was not mentioned (Renko et al., 2007).

The symptoms of PFAPA syndrome are reported to appear less frequently and milder after lasting for years and especially before spontaneous healing (Adachi et al., 2011; Colotto et al., 2011; Rigante et al., 2017; Vitale et al., 2016).

2.3 Differential diagnostics

Fever is a common cause of physician visits in children; approximately 31% of all contacts with general practitioners are associated with fevers, (De Bont et al., 2015) but recurrent fevers are rare. Recurrent fevers can be classified into intermittent fevers and periodic fevers. In periodic fevers, patients have clockwork repeated episodes of fevers with healthy periods between, whereas in intermittent fevers the disease cycle does not reoccur so promptly and regularly. A symptom diary is the main tool to catch the pattern of periodicity and symptom profile of the disease and separate PFAPA from other recurrent fever diseases (Marshall, 2013).

2.3.1 Periodic fevers

The classic periodic fever syndromes are PFAPA and cyclic neutropenia. Cyclic neutropenia is a rare genetic disease due to mutations in the gene for neutrophil elastase. It is mostly inherited in an autosomal-dominant fashion, but sporadic mutations are also possible. It is approximated to affect one in every million people in the general population. The patients suffer from periodic fevers after every 21 days and blood neutrophil levels oscillate, being near zero for several days during the cycle. Painful mouth ulcers, cellulitis, and other invasive infections may be associated with the disease. Cyclic neutropenia can be diagnosed with repeated, weekly neutrophil accounts, samples from bone marrow, and genetic tests (Dale & Welte, 2011).

2.3.2 Intermittent fevers

Intermittent fevers may mimic periodic fevers and must be taken into account in differential diagnostics. The most common cause for intermittent fevers in pediatric patients is common viral infections. Children of preschool age may have on average five, but even up to 11 symptomatic respiratory infections annually, often
accompanied with fevers. (Grüber et al., 2008; Byington et al., 2015; Toivonen et al., 2016).

Although repeatedly occurring infections may mimic PFAPA, they differ from PFAPA syndrome with the irregularity of fevers and with various local signs and symptoms of infections. Malaria and other tropical infections may also cause recurrent fevers, but seldom have PFAPA-like periodic fevers and are often suspected due to history (Marshall, 2013).

One of the most common differential diagnostic challenges of repeatedly occurring infections is recurrent tonsillitis. It is a common entity, with the lifetime prevalence estimated to be 12% (Kvestad et al., 2005). The symptoms of tonsillitis may be identical of those of a PFAPA flare with fever, cervical lymphadenitis, and the clinical picture of tonsillopharyngitis; a single episode of tonsillitis is not always distinguishable from a PFAPA episode. The main difference between recurrent fevers and PFAPA syndrome is the periodic pattern of fevers in PFAPA (Hofer et al., 2014; Thomas et al., 1999).

Monogenic autoinflammatory fever syndromes like Familial Mediterranean Fever, (FMF), tumor necrosis factor receptor-associated periodic syndrome, (TRAPS), Cryopyrin-associated periodic syndrome (CAPS) and hyperimmunoglobulinemia D (HIDS) are important entities in differential diagnostics of PFAPA, but their role in practical diagnostics depends on the ancestry of a population (Koné-Paut et al., 2016; Koyfman et al., 2013; Marshall, 2013).

For example, the prevalence of Familial Mediterranean Fever, FMF, is approximated to be more than one in 256 in some parts of the Middle East, but the disease has very rarely been reported in Finland (Ben-Chetrit & Levy, 1998; Korppi et al., 2003; Pettersson et al., 2006). Most of the autoinflammatory fever syndromes are genetic diseases, and some cluster strongly in families. The fevers are mainly not strictly periodic, but in the beginning of the symptoms the rhythm of the disease is not easy to detect, and other symptoms and findings may mimic PFAPA. Autoinflammatory syndromes are mainly chronic and progressive diseases with significant morbidity (Grateau et al., 2005; Ostring & Singh-Grewal, 2016).

Table 2 lists the features of the most important differential diagnostic monogenic diseases.
<table>
<thead>
<tr>
<th>Disease Feature</th>
<th>Inheritance pattern</th>
<th>Ethnicity</th>
<th>Age of onset</th>
<th>Duration of attacks (days)</th>
<th>Clinical findings</th>
<th>Amyloidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMF</td>
<td>Inflammasomopathia</td>
<td>Autosomal recessive</td>
<td>Mediterranean</td>
<td>&lt; 20</td>
<td>1–3 Serositis, erysipelas erythema, abdominal or chest pain</td>
<td>Yes</td>
</tr>
<tr>
<td>TRAPS</td>
<td>Inflammasomopathia</td>
<td>Autosomal dominant</td>
<td>European</td>
<td>&lt; 20</td>
<td>&gt; 7 Abdominal pain and periorbital edema, rash, splenomegaly</td>
<td>Yes</td>
</tr>
<tr>
<td>CAPS</td>
<td>Inflammasomopathia</td>
<td>Autosomal dominant/sporadic</td>
<td>European/any</td>
<td>&lt; 1–20</td>
<td>1–3/continuous Conjunctivitis, deafness, headache, nausea, rash, arthropathy</td>
<td>Yes/No</td>
</tr>
<tr>
<td>HIDS</td>
<td>Inflammasomopathia</td>
<td>Autosomal recessive</td>
<td>European</td>
<td>&lt; 1</td>
<td>3–7 Rash, adenopathy, serositis, vomiting, diarrhea, headache</td>
<td>No</td>
</tr>
</tbody>
</table>
2.4 Epidemiology

PFAPA syndrome occurs both among males and females and a minor male predominance is seen in most of the published cohorts (males 55–65%) (Feder & Salazar, 2010; Hofer et al., 2014; Tasher et al., 2006; Thomas et al., 1999). The syndrome has been diagnosed among patients from different ethnic backgrounds in Asia, Africa, Middle East, America, and Europe (Feder & Salazar, 2010; Hofer et al., 2014; Thomas et al., 1999).

The only published estimate of the incidence of PFAPA syndrome comes from Norway and is reported to be 2.3/10 000/year children up to five years of age, making PFAPA the most common pediatric periodic fever syndrome (Førsvoll et al., 2013).

2.5 Etiology and pathogenesis

2.5.1 Systemic inflammatory responses

At the time of a fever flare, PFAPA patients are very ill. Acute-phase parameters like the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels, and leukocyte counts increase. Leukocytosis is due to elevated neutrophil and monocyte counts (Brown et al., 2010; Brown et al., 2010; Forsvoll & Oymar, 2007; Sundqvist et al., 2013; Valenzuela et al., 2013). Serum amyloid A (SAA) levels are high at the time of PFAPA fevers, but decrease during the flares unlike in many autoinflammatory fever syndromes (Brown et al., 2010; Sundqvist et al., 2013). The serum procalcitonin level remains low throughout the whole period (Brown et al., 2010; Kraszewska-Glomba et al., 2016; Yazgan et al., 2012b; Yoshihara et al., 2007). Between fever flares, the complete blood count and ESR are usually normal, as well as quantitative immunoglobulin levels (Feder & Salazar, 2010; Renko et al., 2007).

In PFAPA syndrome, the levels of proinflammatory cytokines like interleukin-6 (IL-6), caspase-1, and IP-10/CXCL10 are elevated at the time of fever flares (Brown et al., 2010; Forsvoll et al., 2015; Kolly et al., 2012; Stojanov et al., 2006; Stojanov et al., 2011).

In addition, the innate immunology functions of neutrophils, apoptosis, and the priming and generation of intracellular oxidative bursts are altered in PFAPA syndrome (Sundqvist et al., 2013). Interleukin 1β (IL-1β) production seem to be dysregulated in PFAPA (Brown et al., 2010; Kolly et al., 2012; Stojanov et al., 2011).
The regulation of IL-1β production is closely related to inflammasomes, intracellular protein complexes that are a crucial part of innate immunity. Inflammasomes can be activated by environmental stimuli, such as the presence of microbes (de Zoete et al., 2014). The immunological profile of PFAPA syndrome refers to autoinflammatory syndromes, (Brown et al., 2010; Forrest et al., 2015; Kolly et al., 2012; Stojanov et al., 2006; Stojanov et al., 2011) which are caused by dysfunction of the innate immunity (Kastner et al., 2010).

At the time of a PFAPA flare, the levels of interleukin-6 and interleukin-18 increase (Brown et al., 2010; Forrest et al., 2015; Kolly et al., 2012; Stojanov et al., 2006; Stojanov et al., 2011).

In addition, the levels of chemokines like IP-10/CXCL10 are raised in fever flares (Brown et al., 2010; Forrest et al., 2015; Kolly et al., 2012; Stojanov et al., 2006; Stojanov et al., 2011; Wekell et al., 2016).

These changes in the cytokine pattern are supposed to be associated with interferon -γ (INF-γ) secretion, the Th-1 differentiation of CD4+ T cells, and the Th1 response (Stojanov et al., 2006). Due to these findings, adaptive immunity might be activated in PFAPA syndrome alongside the innate immune system (Wekell et al., 2016). Therefore, behind the dysregulation of innate immunity in PFAPA syndrome could be an environmental trigger, for example microbes (Stojanov et al., 2011). As the cytokine profile of PFAPA indicates an activation of innate immunity, it also reveals an activation of adaptive immunity at the time of a fever flare.

### 2.5.2 Local findings in the pharynx

The tonsils/pharynx plays a crucial role in PFAPA. The most classical clinical findings of PFAPA are in the pharyngeal area, and the only known curative treatment is TE or TEA, implicating the palatine tonsils in the pathogenesis of PFAPA. The palatine tonsils are a part of the oropharyngeal mucosal-associated lymphoid tissue zone called Waldeyer’s tonsillar ring (Moore, 1992). They are the part of the first line defense of the human immune system. The immunological reactions in the tonsils against pathogens are based on their anatomical location and surface structure, making the introduction of pathogens for tonsillar immunological mechanisms effective. A close relation of the mucous membrane with B- and T-cell rich tonsillar tissue, as well as unique composition of microbes of the epithelium, are the essential parts of the immunological defense of the tonsils. The interaction between the mucous microbes and the underlying lymphoid tissue in the tonsils.
may be important in the activation of inflammatory reactions (Brandtzaeg 2011; Fossum et al., 2017).

Despite the important immunological role of tonsils, it seems that tonsillectomy has no clinically significant negative effect on the immune system (Bitar et al., 2015). Tonsillectomy is an effective treatment in several pharyngeal diseases like recurrent pharyngitis (Koskenkorva et al., 2013), and also it seems to be beneficial in treating diseases like psoriasis or IgA nephropathy (Yokoyama et al., 2004; Duan et al., 2017; Thorleifsdottir et al., 2017).

Few studies have focused on the role of microbes in PFAPA. The throat cultures of all 22 PFAPA patients in a study by Førsvoll et al remained negative (Førsvoll et al., 2013). The white patches of the tonsils during the fever flares were gram stained and cultured, but revealed polymorphonuclear leucocytes and normal respiratory bacterial flora. In the molecular analysis of removed PFAPA tonsils, nucleic acids of respiratory syncytial virus A and B, influenza viruses A and B and parainfluenza viruses 1, 2, and 3 or bacteria atypical for pharyngeal area were not found (Pignataro et al., 2009).

As most of the micro-organisms in nature are not amenable to culture, the detection of microbes of these communities is made using molecular, genome-enabled methods (Proctor, 2016). With those methods it is possible to detect all the community of commensal, symbiotic, and pathogenic micro-organisms, called microbiota. The genome of micro-organisms in microbiota is called microbiome (Lederberg & McCray, 2001; Turnbaugh et al., 2007). Bacterial microbiome is shown to be rich in the oropharyngeal region (Segata et al., 2012).

The only study concerning the microbiota of PFAPA patients has been published as an abstract by Freeman et al. (2014). They used next-generation sequencing to analyze bacteria and viruses of punch biopsy samples of six PFAPA patients and eight controls but found no differences in bacteria or virus types between groups. Though, in other tonsil related diseases like recurrent tonsillitis, microbiota of tonsillar tissue differs significantly from that of controls (Jensen et al., 2013).

Complex microbial communities called biofilms are common in the pharyngeal area (Woo et al., 2012). The microbes of biofilms are able to communicate with each other creating dynamic microbial aggregates. Biofilms are very resistant against antimicrobials and protect themselves effectively from the immune system (Majumdar & Pal, 2018). Infections caused by biofilms are most effectively treated by removing the affected focus. Biofilms seem to play a crucial role in recurrent tonsillitis and tonsillar hypertrophy, explaining the effectiveness of the TE as a
treatment option of those diseases (Diaz et al., 2011a; Galli et al., 2007; Torretta et al., 2013; Drago et al., 2011). The role of biofilms in PFAPA syndrome is not known.

The palatine tonsils are lymphoid tissue structures covered with stratified squamous cell epithelium. The surface of the tonsils is spotted with crypts invaginating deep in the lymphatic tissue, allowing a close relationship between the lymphatic tissue and oropharyngeal microbes (Perry, 1994). Palatine tonsils contain lymphoid nodules with germinal centers, where proliferating B cells are located in the center and resting B cells and T cells in the periphery (Standing, 2008). The histology of palatine tonsils in PFAPA syndrome between flares has been reported to represent a feature of chronic tonsillitis, and no particular abnormality in the architecture or amounts of B- and T-cells has been found in immunohistochemical staining (Manthiram et al., 2017a; Peridis et al., 2010a). In Førsvolls’ study, the amount of CD8+ cells in the germinal centers of tonsils was higher in PFAPA patients than in those of the controls (Førsvoll et al., 2015).

The size of germinal centers of the PFAPA tonsils have shown to be smaller than the tonsils of the controls, who suffered from obstructive sleep apnea. In PFAPA patients, squamous epithelia have reported to be wider than in the controls (Manthiram et al., 2017a).

Dytrych et al. (2015) found that the percentage of B-lymphocytes of tonsils was lower than in the controls operated due to obstructive reasons, but cytotoxic CD8pos T-lymphocytes were approximately two-fold higher. The naïve stages of both CD4pos and CD8pos T-lymphocytes were also increased. The expressions of CXCL10, CXCL9, and CCL19 were higher in PFAPA tonsils. These findings may indicate that the uninhibited, polyclonal response of newly derived lymphocytes may have a role in PFAPA syndrome.

2.6 Genetics

PFAPA syndrome was thought to be a sporadic disease until the first reports of familial cases were published (Adachi et al., 2011; Antón-Martin et al., 2011; Sampaio et al., 2009; Valenzuela et al., 2009) and the first large interview study of 84 PFAPA patients revealed clear clustering in families (Cochard et al., 2010). Reports of family members having PFAPA syndrome, recurrent fevers, or recurrent tonsillitis have varied from 17 to 78% in the literature (Akelma et al., 2013; Førsvoll et al., 2013; Hofer et al., 2014; Manthiram et al., 2016; Perko et al., 2015).
Published pedigrees of families with PFAPA syndrome have features of autosomal dominant inheritance pattern (Di Gioia et al., 2015; Manthiram et al., 2016).

The pathogenesis and the genetic basis of the other autoinflammatory fever syndromes is known, and therefore the screening of mutations in PFAPA has mainly focused on the gene variants causing those syndromes; gene MEFV (FMF), gene TNFRSF1A (TRAPS), gene MVK (HIDS), gene NLRP3 (CAPS), and NOD2/CARD15. Any mutations or variants in these genes have not found to be associated with PFAPA syndrome (Batu et al., 2016; Chandrakasan et al., 2014; Dagan et al., 2010; Perko et al., 2015), but such variants may modify the phenotype of the syndrome (Berkun et al., 2011; Taniuchi et al., 2013). PFAPA patients with one or more variation or mutation in the MVK, TNFRSF1A, or MEFV genes have been reported to have more abdominal symptoms, cutaneous rash, and arthralgia than PFAPA patients without these variations or mutations (Gattorno et al., 2009). Patients with variants in AIM2, NLRP3, or MEFV genes had an earlier disease onset, shorter fever phases, and more often the classic triad of aphthous stomatitis, adenitis, and pharyngitis (Perko et al., 2015). Whole genome sequencing did not indicate any mutated gene causing the syndrome. It is very unlikely, that associations between genes involved with genetic periodic and intermittent fevers and PFAPA might exist. PFAPA appears to be a complex disease of oligogenic or polygenic inheritance (Di Gioia et al., 2015).

2.7 Risk factors of PFAPA syndrome

The risk factors of PFAPA syndrome are not known. The only suggested risk factor for PFAPA syndrome is vitamin D deficiency (Mahamid et al., 2013; Stagi et al., 2014), and no other controlled studies about the risk factors of the syndrome have been published. The study of Thomas et al. (1999) report, that in their cohort of 94 PFAPA patients, consistent complications of pregnancy or delivery were not seen, and no trends in the occupations of parents were found, but the study design was not controlled.
2.8 Treatment options for PFAPA syndrome

2.8.1 Pharmacological treatments

Antibiotics and non-steroidal anti-inflammatory agents are ineffective in PFAPA syndrome, but glucocorticoids are effective in treating fever flares (Padeh et al., 1999; Peridis et al., 2010b; Ter Haar et al., 2013; Thomas et al., 1999; Wurster et al., 2011a). A single dose of steroids offer a rapid resolution of fever for most patients, and only 5% of the patients are non-responders (Hofer et al., 2014; Krol et al., 2013). Glucocorticoids cannot prevent upcoming episodes and may shorten the interval between fevers (Thomas et al., 1999; Feder & Salazar, 2010). The usage of short-course corticosteroids is associated with adverse reactions, mainly vomiting, behavioral changes, and sleep disturbances in children (Aljebab et al., 2016). In PFAPA patients, side effects due to corticosteroid treatment are common; 15–35% of 54 PFAPA patients reported side effects, mainly restlessness (Tasher et al., 2006; Yazgan et al., 2012a).

Colchicine is an inflammation-reducing medication with an unknown mechanism of action. It is used as a prophylactic treatment of FMF and therefore studied in PFAPA patients as well. It seems to increase the interval between fever attacks among some PFAPA patients and is occasionally used as a prophylactic treatment of the syndrome (Butbul Aviel et al., 2016; Dusser et al., 2016; Gunes et al., 2017; Padeh et al., 1999; Tasher et al., 2008). Cimetidine, a common H2 antagonist with immune-modulating properties, might have reduced symptoms in some PFAPA patients. No controlled trials exist, and its use is rare according to the report of the Eurofever registry (Feder & Salazar, 2010; Ter Haar et al., 2013; Thomas et al., 1999; Wurster et al., 2011b).

Some preliminary and experimental medical treatments of PFAPA syndrome have been reported to have interesting results, although their clinical significance is currently insignificant. Stojanov et al tested anakinra for five PFAPA patients at the time of fever flares with good results. As a recombinant IL-1R antagonist, anakinra confirms the essential role of IL-1 in the pathogenesis of PFAPA syndrome, which has been suspected earlier based on the cytokine profile of the disease (Stojanov et al., 2006; Stojanov et al., 2011). Vitamin D and pidotimod, an immunomodulatory agent with activity on both innate and adaptive immune responses, have promising preventive effects for PFAPA patients by relieving the symptoms and frequency of the flares (Buongiorno & Pierossi, 2015; Stagi et al., 2014). Preliminary observations of the administration of the oral probiotic strain
Streptococcus salivarius (K12) for four PFAPA patients reduced all their signs and symptoms and offered full remission for three of them. Modifying the microbiological balance of the mucous membranes of the pharynx may have a key role in the pathogenetic mechanisms of PFAPA syndrome (Di Pierro et al., 2016).

2.8.2 Operative treatments

The first case report of four PFAPA patients successfully treated by TE/TEA was published in 1989 (Abramson et al., 1989). Thereafter, two randomized controlled trials and a Cochrane review about TE/TEA as a treatment for PFAPA syndrome has been published (Garavello et al., 2009; Renko et al., 2007). All 14 patients in Renko’s study and 12/19 patients in Garavello’s study were cured after TE. TE/TEA is shown to be a very effective treatment for the syndrome. (Burton et al., 2014; Garavello et al., 2009; Renko et al., 2007; Forsvoll & Oymar, 2018). In the study by Erdogan et al. (2016), and in the meta-analysis by Peridis et al. (2010b), surgical treatment of PFAPA was superior to medical treatments.

The role of adenoidectomy combined with tonsillectomy in treating PFAPA syndrome is unclear. In Garavello et al’s study, all patients underwent TEA, but the patients in Renko et al’s study either had TE or TEA, and both operations were effective (Burton et al., 2014; Garavello et al., 2009; Renko et al., 2007). Adenoidectomy without tonsillectomy was ineffective in three patients in the cohort of Thomas et al, (1999), and no other studies on solely adenoidectomy have been published.

Long-term follow-up studies after TE/TEA are few. Most describe the observation of a small proportion of larger PFAPA cohorts, operated on due to having a severe form of the disease or poor response to medical treatment (Padeh et al., 1999; Pignataro et al., 2009; Thomas et al., 1999; Wong et al., 2008). Ligamelli et al. (2012) reported, that 97% of their 102 PFAPA patients went into complete remission immediately after the operation, and it sustained after a mean 3.6 years of follow up. A Turkish prospective serie of 23 PFAPA patients reported constant remission after TE/TEA in 91% cases in a one year follow up study (Aktas et al., 2017).

The most common risk of TE/TEA is peri- or postoperative hemorrhage. On average, 0.8–2.7 % of the children under the age of 11 experience postoperative bleeding after tonsil surgery (Harounian et al., 2016; Hessén Söderman et al., 2011). The risk of postoperative bleeding is lowest in the younger children and serious postoperative bleedings are rare. The prospective audit of 33 921
tonsillectomies in Great Britain revealed one lethal complication (Royal College of Surgeons of England, May 2005).

2.9 Long-term health of PFAPA patients

PFAPA syndrome is considered an autoinflammatory disorder as well as HIDS, TRAPS, and FMF, which have a progressive nature and are associated with remarkable co-morbidities by adulthood (Cattalini et al., 2016). PFAPA is also a chronic disease, but has spontaneous healing potential (Hofer et al., 2014; Wurster et al., 2011b). No systematic or controlled trials exist on the long-term health of PFAPA patients after pharmacological or operative treatments.

Wurster et al observed that 20% of the 60 PFAPA patients in their study reported symptoms of allergic rhinitis, 10% eczema and 8.3% food or drug allergies. Of their patients, 28% were hospitalized for other reasons than PFAPA along the follow-up period ranging from 12 to 21 years (Wurster et al., 2011b). In the earlier, 10-year follow-up study of the same cohort, four PFAPA patients were reported to have neurobehavioral problems. The children of the cohort were reported to have fewer common infections than their siblings, (Thomas et al., 1999) as were the patients in Feder & Salazar’s (2010) cohort. In these cohorts, the patients were mainly treated with conservative methods (Feder & Salazar, 2010; Thomas et al., 1999; Wurster et al., 2011b). The only case-control study on the health of PFAPA patients compared the infections, recurrent otitis media, and tympanostome tube insertion frequencies between PFAPA patients and controls recruited from primary health care. No differences were found between the groups (Manthiram et al., 2016). The long-term health of PFAPA patients after TE/TEA is not known.

As normal growth is most often defined as one of the diagnostic criteria, it has not been separately studied or published in the literature. In a cohort of 25 PFAPA patients the height and body mass index (BMI) were similar to 111 controls, (Stagi et al., 2014) but no other studies exist on the growth of PFAPA patients.
3 Aims of the present study

The aims of this thesis were as follows:

1. To assess and compare the long-term outcome of PFAPA patients treated by TE or TEA with a classic or incomplete phenotype.
2. To compare the health, comorbidities, and growth of PFAPA patients with healthy controls.
3. To compare the histological and microbiological findings of the tonsils of PFAPA patients with controls with conventional and modern sequencing technologies.
4 Patients and methods

4.1 Setting

Oulu University Hospital, Oulu, Finland, serves a population of 405,635 people (Statistics Finland December 31, 2014). It is the only hospital taking care of pediatric patients in the area and thus diagnosing and treating the vast majority of childhood periodic fever syndromes. Approximately 90% of all the pediatric TEs in the area take place in the Department of Otorhinolaryngology of Oulu University Hospital. The treatment of PFAPA syndrome in Oulu University Hospital has been TE with or without adenoidectomy since the first published series of four PFAPA patients treated effectively by TE (Abramson et al., 1989). The main technique used has been cold dissection with monopolar electrocauterization to obtain hemostasis. The technique used in removing adenoids has been adenoidectomy. Corticosteroids, cimetidine, or colchicine has not been used as a treatment for PFAPA syndrome.

4.2 Study populations

4.2.1 Follow-up cohort (studies I and II)

The aim was to identify all possible PFAPA children who underwent TE/TEA before the age of 12 at Oulu University Hospital between 1987 and 2007. Since PFAPA syndrome does not have a specific ICD code, the history of all the children operated on due to recurring fevers were reviewed. Diagnostic criteria of PFAPA syndrome used in the studies of the theses were: 1) at least five regularly occurring, strictly periodic high-fever flares, 2) healthy periods between fevers, 3) the exclusion of other diseases in otherwise healthy children with possible PFAPA signs, including aphthous stomatitis, pharyngitis, and adenitis. Genetic autoinflammatory fever syndromes were not demanded to be tested without a known family history of FMF, TRAPS, CAPS, or HIDS due to the rarity of the diseases in the Finnish population (Korppi et al., 2003; Pettersson et al., 2006). The regularity of fevers had to be mentioned by an otorhinolaryngologist or pediatrician in the hospital medical records.

A total of 3,852 children under 12 years of age had undergone TE/TEA between the years 1987 and 2007, of which 3,030 children were excluded, as they had been
operated on because of other definite diagnosis: tonsillar hypertrophy, peritonsillar abscess, or recurrent *Streptococcus pyogenes* throat infection. The medical records of all remaining 825 children were reviewed and 132 fulfilled PFAPA inclusion criteria (Figure 1), 70% (93/132) had been operated due to diagnosed PFAPA syndrome and 30% (39/132) were diagnosed as PFAPA retrospectively by the research group via medical records. PFAPA was the reason for 3% of all pediatric TEs in our hospital.

![Bar chart showing number of operated PFAPA patients per year from 1987 to 2007.](image)

**Fig. 1.** TE/TEAs performed in Oulu University Hospital due to PFAPA syndrome between years 1987–2007, total n = 132.

Altogether 132 patients who met the inclusion criteria were invited for a study visit in autumn 2011. Of them, 94 (71%) attended the visit, where they underwent a clinical examination by a physician and either they or their parents completed a detailed questionnaire concerning PFAPA symptoms and long-term postoperative health. In addition, 23 patients were interviewed by phone and two further patients returned completed questionnaires. Four patients did not wish to take part in the study, eight could not be reached, and one had died in a drowning accident. Thus, postoperative follow-up data was received on 119 PFAPA cases (90%). All the patients’ diagnoses were confirmed to be PFAPA based on medical records, questionnaires, and/or interviews (Figure 2).
Fig. 2. Flow chart illustrating how the study populations of studies I and II were obtained.
In study I, the main outcome measure was the long term effectiveness of TE in PFAPA patients. The 119 patients were divided into two groups based on their medical history; those who met the classic Thomas’ diagnostic criteria (Classic PFAPA) (Table 1) and those who did not and had an incomplete PFAPA along the Thomas’ criteria (Incomplete PFAPA). Patients with incomplete PFAPA had suffered from at least five regularly recurring fever episodes, but lacked the symptoms of aphthous stomatitis, pharyngitis, or adenitis, or were over age five at the onset of symptoms, or both. The classic PFAPA criteria were met by 58/119 (49%) patients, and 50/119 (42%) had incomplete PFAPA. In 11/119 (9%) patients, the information on the onset age or oropharyngeal symptoms during fever flares was not reported; thus, 108 PFAPA patients were included in Study I. At the onset of the fever episodes, 87 patients (81%) were under the age of five years and 21 (19%) were older; 74 (69%) patients had presented with aphthous stomatitis, pharyngitis, or adenitis during the fever episodes, while the remaining 34 (32%) cases had presented with regularly recurring fever as the only symptom.

In study II, the primary hypothesis was that PFAPA syndrome might be associated with other diseases and disorders of the immune system. To study the health of PFAPA patients, at least two sex-, age-, and birth place-matched controls were aimed to be found for the 119 PFAPA patients. Randomly selected controls were collected from the database of the Population Register Center of Finland. A questionnaire was mailed to 915 prospective controls, and 202 completed questionnaires were received. To ensure at least one control for each PFAPA patient, 28 of the original 915 controls who had not responded to the mailed questionnaire were contacted by telephone. Finally, 230 control subjects were enrolled (Figure 2). In the final study population, 1/119 (0.8%) PFAPA patient had four controls, 16/119 (13%), had three controls, 75/119 (63%) had two controls, and 27/119 (22%) had one control. All the controls were interviewed by phone (28/230, 12%) or mail (202/230, 88%).

The ethnic background of the patients and controls in all studies was Caucasian, North European. The male gender was slightly more predominant in all studies (Table 3).

The mean age of the 119 PFAPA patients was 2.7 years (SD 2.4) at the onset of the symptoms, 4.4 years (SD 2.6) at the time of TE/TEA, and 13.2 years (SD 5.5) at the time of the clinical study visit or telephone interview. The mean follow-up period from TE/TEA to the study visit or interview was 9.0 years (SD 4.7) (Table 4). A total of 82 patients (69%) underwent TEA and 37 (31%) patients TE.
Table 3. Gender and age profile of study populations in studies I–IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>Gender (boys)</th>
<th>Age at the time of TE, y mean (SD)</th>
<th>Age at the onset of periodic fevers, y mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic PFAPA (n = 58)</td>
<td>36 (62%)</td>
<td>3.3 (1.5)</td>
<td>1.8 (1.2)</td>
</tr>
<tr>
<td>Incomplete PFAPA (n = 50)</td>
<td>31 (62%)</td>
<td>5.3 (3.1)</td>
<td>3.8 (3.0)</td>
</tr>
<tr>
<td>Study II:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFAPA patients (n = 119)</td>
<td>79 (66%)</td>
<td>4.4 (2.6)</td>
<td>2.7 (2.4, n = 113)</td>
</tr>
<tr>
<td>Controls (n = 230)</td>
<td>140 (61%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study III and IV1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFAPA patients (n = 31)</td>
<td>18 (58%)</td>
<td>3.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Controls (n = 24)</td>
<td>8 (33%)</td>
<td>5.8</td>
<td></td>
</tr>
</tbody>
</table>

1 In study IV, the number of PFAPA patients was 30

Table 4. Characteristics of the PFAPA patients in the study II (n = 119).

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean (SD)</th>
<th>n (%)</th>
<th>total n with data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset of PFAPA fevers, years</td>
<td>2.7 (2.4)</td>
<td></td>
<td>113</td>
</tr>
<tr>
<td>Age at the time of TE/TEA, years</td>
<td>4.4 (2.6)</td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>Age at the time of the study visit/interview, years</td>
<td>13.4 (5.5)</td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>Follow-up time from the onset of the syndrome to the study visit/interview, years</td>
<td>10.4 (4.9)</td>
<td></td>
<td>113</td>
</tr>
<tr>
<td>Follow-up time after TE/TEA, years</td>
<td>9.0 (4.7)</td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>Length of PFAPA cycle, days</td>
<td>27.6 (9.2)</td>
<td></td>
<td>113</td>
</tr>
<tr>
<td>Duration of fever flare, days</td>
<td>4.1 (1.3)</td>
<td></td>
<td>107</td>
</tr>
<tr>
<td>Highest measured body temperature °C</td>
<td>39.3 (0.7)</td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>Onset of the syndrome under the age of five years</td>
<td>91 (80.5%)</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Aphthous stomatitis at the time of fever</td>
<td>31 (27.2%)</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Pharyngitis at the time of fever</td>
<td>69 (59.5%)</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Adenitis at the time of fever</td>
<td>62 (52.5%)</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Fever as the only symptom of PFAPA</td>
<td>24 (31.2%)</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Pauses in the syndrome before TE/TEA</td>
<td>23 (20.5%)</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

4.2.2 Microbiological and histological studies (studies III and IV)

Altogether 31 consecutive children who underwent TE due to PFAPA syndrome between March 2006 and April 2010 were recruited to a research project studying the histology and microbiology of the removed tonsils. The diagnostic criteria were those earlier described. During the same period, 24 children undergoing TE due to
obstructive sleep apnea and hypertrophied tonsils served as controls. Fifteen PFAPA patients in studies III and IV were also study patients in studies I and II.

4.3 Study visit and clinical examination (studies I and II)

The 94 PFAPA patients participating the study visit were medically examinated. Examinations included ear-, nose-, and throat status, the auscultation of the heart and lungs, and the inspection of the skin. Height, weight, blood pressure, pulse, and the body temperature were measured at the study visit. The 23 patients who did not attend the study visit and controls reported the latest measured values of height, weight, and blood pressure.

4.4 Questionnaires

4.4.1 Studies I and II

To evaluate possible differences in subsequent health between the PFAPA patients and controls, the questionnaire covered not only PFAPA symptoms, but general health before and after TE. Data on diseases other than PFAPA that required consulting a physician, visiting a hospital or taking regular medication were collected both before and after TE/TEA. The questionnaire asked specifically about a family history of regularly recurring fevers in 1st degree relatives. In 117/119 (98%) PFAPA patients and 195/226 (86%) controls, the questionnaires were completed with the assistance of parents.

The data on the patients and controls on physician and hospital visits due to diagnosed or suspected infections, allergies, and autoimmune diseases were collected, in addition to data on the use of antibiotics. Also data on other diseases, symptoms, regular medications, and surgical histories were collected. To set apart symptoms and signs of PFAPA syndrome, data on hospital visits by the PFAPA patients before and after the TE/TEA were recorded separately. Hospital visits were also analyzed separately to avoid recall bias, as diseases or conditions demanding hospital visits might be more memorable. For the analyses, the reported causes of physician or hospital visits were grouped into seven main categories (infections, autoimmune diseases, traumas, migraine, asthma, neurological problems, and any kind of tumor). To estimate the social class of the family, the parents were asked to give their occupations in the questionnaire, and the occupations were then grouped
into seven categories. The social class of the family was determined by the occupation of the father.

With the assistance of their parents, the cases and controls estimated their general health on a Likert scale as 0–10, where 0 was the poorest possible health and 10 denoted excellent health. The new Finnish age- and gender-specific growth standards for children and adolescents aged 0–20 years was used to transform the length measurements into age- and gender-specific $z$-scores. Weight was assessed as the relative percentage difference from the gender-specific median weight-for-height (Saari et al., 2011).

### 4.4.2 Studies III and IV

The data on the children’s symptoms before their surgery were collected from hospital records and the questionnaires. The onset of the syndrome and the profile of fevering were assessed in detail.

### 4.5 Previous use of antimicrobials

In studies I and II, the lifetime usage of antibiotics and the age at the time of the first antibiotic treatment were assessed in questionnaires. As previous use of any antimicrobials before TE might have an influence on the microbes of tonsils in studies III and IV, the data of the antimicrobials the patients had purchased within 12 months before TE was obtained from the Finnish national Drug Purchase Register maintained by the Social Insurance Institution of Finland (KELA).

### 4.6 Tonsillar samples (studies III and IV)

#### 4.6.1 Histologic examinations (study III)

The removed tonsils were fixed in formalin and embedded in paraffin, cut into 5-µm thick sections, and stained with hematoxylin and eosin. Our focus was on active inflammation (microabscesses and crypt-infiltrating neutrophils) and possible findings of Actinomyces-like organisms along with routine histologic evaluation.
4.6.2 Conventional microbiological examinations (study III)

Fresh tonsil samples were cultured for bacteria, mycobacteria, yeasts, and viruses by conventional methods. The polymerase chain reaction (PCR) was used to find 13 different viruses from the tonsil samples; parainfluenza-, influenza-, respiratory syncytial-, entero-, rhino-, adeno-, metapneumo-, herpes simplex-, varicella zoster-, cytomegalo-, Epstein Barr- and human papilloma viruses. Also human herpesvirus 6 was detected. The results of all these microbial techniques were combined for the analyzes and categorized the microbes as either present or absent.

4.6.3 Detection of biofilms (study III)

Biofilms of the tonsil surface were visualized with a scanning electron microscope (SEM) and categorized as either present or absent (Figure 3).
Fig. 3. Scanning electron microscope pictures of tonsil surface of patients of study III A) with and B) without biofilm formation.

A tissue sample was first rinsed. It was then oriented and fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer. After fixation, the sample was briefly rinsed with phosphate-buffered saline and then with sterilized aqua. The specimen
was dehydrated by placing it in ethanol in series. Subsequently, the specimen was processed with a Critical Point Dryer CPD 030 (Bal-Tec) and given a platinum coating with a High-Resolution Sputter Coater (Agar). All samples were evaluated with a Zeiss Ultra plus field emission scanning electron microscope (FESEM) at the Center of Microscopy and Nanotechnology at the University of Oulu by two trained evaluators, who were blinded to indications for TE.

4.6.4 Bacterial microbiota of PFAPA tonsils (study IV)

With the same samples that were used in study III, we continued to study the bacterial microbiota with next-generation sequencing technology to also identify pathogens that were difficult to culture. DNA was extracted from 30 PFAPA tonsil samples and 24 control samples using the DNeasy Blood & Tissue Kit (Qiagen, USA). PCR reactions were performed and DNA purified. For sequencing, each individual sample was pooled in equivalent amount, and the final DNA concentration was measured on a Bioanalyzer DNA chip.

To characterize the microbiomes of tonsillar tissue samples, the hypervariable regions V4-V5 of 16S rRNA genes were sequenced using Ion Torrent. The final dataset consisted of 2.44 million reads after filtering out low-quality and chimeric reads from 54 samples, with a median of 43 638 reads per sample. The reads were clustered into species-level operational taxonomic units (OTUs) to assess the shared diversity among the samples. All samples were rarefied to 24 377 sequences prior the out-based analysis. Each representative OTU was assigned to a taxonomic lineage using the RDP Bayesian classifier.

The rarefaction analysis was carried out to compare the bacterial species richness between samples against the sequencing effort and between the cases and controls. The species richness of PFAPA and control samples was considerably high as there were a total of 3 252 OTUs observed in the complete dataset. The number of OTUs observed in the community varied substantially from 560 to 1 316, signifying highly complex community structure. The Shannon and Simpson index varied from 2.30 to 5.73 and 0.41 to 0.95, respectively, indicating the overall high bacterial diversity in the community.
4.7 Ethics

The Regional Ethics Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland approved the protocol for these studies. All the patients and controls and/or their parents gave their written informed consent for the studies.

4.8 Statistical methods

Stats Direct 2.7.9 and IBM SPSS Statistics vs 18.0 and 22.0 were used to perform the statistical analyses. Proportions were compared between the PFAPA patients and controls. The absolute differences and their 95% confidence intervals (CIs) were calculated and the standard normal deviate test (SND) was used to analyze the statistical significance of differences between proportions. For continuous variables, means and standard deviations (SDs) or medians, with ranges, were calculated. To test the statistical significance of the differences in continuous variables, a t-test or Mann–Whitney U-test was used, depending on the distributions. The occurrences of diseases in the cases and controls in study II were compared using a univariate logistic regression model, adjusted for the participant’s age at the time of the data collection and the social class of the family, as determined by the occupation of the father, and adjusted odds ratios (ORs) with their CIs and adjusted p-values are reported.

In studies III and IV, the child’s age could have influenced the microbiology of the tonsils and because the sample size was too small to allow analyses on age groups, forward stepwise logistic regression analyses adjusted for age and sex, were performed. ORs and CIs for individual microbes were calculated.
5 Results

5.1 The classic and incomplete PFAPA syndrome (study I)

All patients with incomplete PFAPA and 97% (n = 58) of patients with classic PFAPA achieved a prompt and constant response for TE in this nine-year follow up. Before the operation, the fever symptoms of PFAPA syndrome were alike. Fever flares had occurred very regularly both in the patients with classic or incomplete PFAPA. The time interval between the first day of two consecutive episodes varied within seven days in 73% of the patients meeting the classical diagnostic criteria and in 87% of those with incomplete PFAPA (p = 0.06). The mean length of fever flares did not differ between the groups. The mean number of febrile days per episode did not differ significantly between the groups either. The mean highest reported body temperature during an episode was 0.3°C higher in children with classic PFAPA than in those with incomplete PFAPA, and the difference was statistically significant (Table 5).
Table 5. Fever profile of patients with the classic or incomplete PFAPA syndrome and p-values for the differences between these groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>The patients with classic PFAPA n = 58</th>
<th>The patients with incomplete PFAPA n = 50</th>
<th>P-value for the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>35 (62%)</td>
<td>31 (62%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Age at the onset of fever episodes, years, mean (SD)</td>
<td>1.8 (1.2)</td>
<td>3.8 (3.0)</td>
<td></td>
</tr>
<tr>
<td>min / max</td>
<td>0.1 / 4.6</td>
<td>0.2 / 10.8</td>
<td></td>
</tr>
<tr>
<td>Age at the time of TE/TEA, years, mean (SD)</td>
<td>3.3 (1.5)</td>
<td>5.3 (3.1)</td>
<td></td>
</tr>
<tr>
<td>min / max</td>
<td>1.3 / 8.8</td>
<td>1.4 / 11.8</td>
<td></td>
</tr>
<tr>
<td>Follow-up time after TE/TEA, years, mean (SD)</td>
<td>6.9 (4.4)</td>
<td>9.0 (4.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>min / max</td>
<td>2.9 / 19.9</td>
<td>2.0 / 20.0</td>
<td></td>
</tr>
<tr>
<td>Number of fever flares prior to TE, mean (SD)</td>
<td>11.5 (6.9)</td>
<td>13.1 (7.7)</td>
<td>0.061</td>
</tr>
<tr>
<td>Prompt and constant response to TE, n (%)</td>
<td>56 (97%)</td>
<td>50 (100%)</td>
<td></td>
</tr>
<tr>
<td>Length of PFAPA cycle, days, mean (SD)</td>
<td>29.1 (9.6)</td>
<td>26.2 (9.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>min / max</td>
<td>7 / 62</td>
<td>11 / 62</td>
<td></td>
</tr>
<tr>
<td>Duration of fever flare, days, mean (SD)</td>
<td>4.3 (1.2)</td>
<td>3.9 (1.3)</td>
<td>0.60</td>
</tr>
<tr>
<td>min / max</td>
<td>2 / 7</td>
<td>1 / 7</td>
<td></td>
</tr>
<tr>
<td>Highest measured body temperature °C at the fever flare, mean (SD)</td>
<td>39.4 (0.5)</td>
<td>39.1 (0.8)</td>
<td>0.014</td>
</tr>
<tr>
<td>Pauses in the fever cycles before TE, (%)</td>
<td>16 (29)</td>
<td>7 (15)</td>
<td>0.087</td>
</tr>
</tbody>
</table>
5.2 Long-term effectiveness of TE(A) to PFAPA

The response to TE was excellent in 114 of 119 (96%) patients. Five patients of all 119 suffered from recurring fevers at the time of the study visit. Two of them responded initially poorly to TE and reported ongoing irregular and nonspecific, mild febrile episodes at the time of the study visit. The other three patients having recurrent fevers at the time of the study visit had responded initially well to TE but had had a relapse of regular periodic fever episodes after several asymptomatic years. Thomas’ criteria captured 53% of the patients responding to TE, while Thomas’ criteria with no age limit identified 80% of the patients responding to TE.

5.3 The health and comorbidity of PFAPA patients

The self-reported general health of the PFAPA patients and controls was good at the time of the follow up (mean 9.1/10 in both groups). In the 94 PFAPA patients who attended the follow-up visit, no acute or chronic diseases not mentioned in their medical histories or reported in the questionnaires were diagnosed. Mean age- and gender-specific z-score of height was -0.2 (SD 1.2, n = 112) among PFAPA patients and -0.2 in the controls (SD 1.08, n = 222). A relative percentage difference from the gender-specific median weight-for-height of PFAPA patients was +5.0% (SD 15.4, n = 112), and in controls +7.5 % (SD 18.6, n = 216), (p = 0.278).

Autoimmune diseases were reported by 3/119 PFAPA patients (2.5%) and 9/230 controls (3.9%), with celiac disease being the most common autoimmune disease (1/119 PFAPA patients and 4/230 controls; p = 0.555) (Table 6). More controls reported allergies (66/230, 29%) than did PFAPA patients (21/119, 18%). However, when adjusted, the difference was not statistically significant (p = 0.626). Pollen allergies were significantly less common in the cases (7/117, 6.0%) than in the controls (39/230, 17%) (p = 0.016). Regarding medication usage, 10/118 (9%) PFAPA patients and 30/230 (13%) controls reported regular usage (p = 0.17). There were no between-group differences in the reported occurrences of any other chronic diseases (Table 6).
Table 6. Numbers and proportions of 119 PFAPA patients and 230 controls with reported current diseases and diagnoses in history, at a mean age of 13.3 years (patients) or 15.7 years (controls). ORs are adjusted for age at data collection and the social class of the family.

<table>
<thead>
<tr>
<th>Cause of physician visit</th>
<th>PFAPA patients n (%) n = 119</th>
<th>Controls n (%) n = 230</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current diseases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen allergy</td>
<td>7 (6.0%, n = 117)</td>
<td>39 (17.0%)</td>
<td>0.347 (0.147 to 0.820)</td>
<td>0.016</td>
</tr>
<tr>
<td>Animal allergy</td>
<td>6 (5.1%, n = 117)</td>
<td>19 (8.3%)</td>
<td>0.725 (0.269 to 1.959)</td>
<td>0.527</td>
</tr>
<tr>
<td>Food allergy</td>
<td>8 (6.8%, n = 117)</td>
<td>22 (9.6%)</td>
<td>0.444 (0.173 to 1.144)</td>
<td>0.093</td>
</tr>
<tr>
<td>Drug allergy</td>
<td>4 (3.4%, n = 117)</td>
<td>10 (4.3%)</td>
<td>0.779 (0.232 to 2.617)</td>
<td>0.686</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td>8 (6.7%)</td>
<td>15 (6.5%)</td>
<td>1.299 (0.480 to 3.147)</td>
<td>0.687</td>
</tr>
<tr>
<td><strong>Autoimmune diseases</strong></td>
<td>3 (2.5%)</td>
<td>9 (3.9%)</td>
<td>1.055 (0.267 to 4.171)</td>
<td>0.939</td>
</tr>
<tr>
<td><strong>Migraine</strong></td>
<td>3 (2.5%)</td>
<td>9 (3.9%)</td>
<td>1.123 (0.274 to 4.595)</td>
<td>0.872</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>115 (99.1%, n = 116)</td>
<td>201 (88.2%, n = 228)</td>
<td>14.819 (1.967 to 111.640)</td>
<td>0.009</td>
</tr>
<tr>
<td>Infections</td>
<td>73 (61.3%)</td>
<td>34 (14.8%)</td>
<td>10.756 (6.121 to 18.899)</td>
<td>0.000</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>50 (42.4%)</td>
<td>17 (7.4%)</td>
<td>0.086 (0.043 to 0.169)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>5 (4.2%)</td>
<td>8 (3.5%)</td>
<td>1.143 (0.351 to 3.721)</td>
<td>0.825</td>
</tr>
<tr>
<td>CNS infections$^1$</td>
<td>3 (2.5%)</td>
<td>1 (0.4%)</td>
<td>10.374 (0.950 to 113.336)</td>
<td>0.055</td>
</tr>
<tr>
<td>Fungal infections</td>
<td>21 (18.1%, n = 116)</td>
<td>16 (7.0%)</td>
<td>3.619 (1.681 to 7.792)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>14 (12.0%, n = 117)</td>
<td>1 (0.4%)</td>
<td>23.904 (3.041 to 187.964)</td>
<td>0.003</td>
</tr>
<tr>
<td>Other fungal infections</td>
<td>7 (6.0%, n = 117)</td>
<td>15 (6.5%)</td>
<td>1.384 (0.509 to 3.763)</td>
<td>0.525</td>
</tr>
<tr>
<td>Physical traumas</td>
<td>13 (10.9%)</td>
<td>14 (6.1%)</td>
<td>2.401 (1.022 to 5.640)</td>
<td>0.045</td>
</tr>
<tr>
<td>Tumors$^2$</td>
<td>4 (3.4%)</td>
<td>6 (2.6%)</td>
<td>1.877 (0.303 to 5.372)</td>
<td>0.739</td>
</tr>
</tbody>
</table>

$^1$ CNS, central nervous system, $^2$ No malignant tumors were reported.
The use of antibiotics during one’s lifetime was reported by almost all the PFAPA patients (115/116, 99%) and by 201/228 (88%) controls (p = 0.009) (Table 6). None of the PFAPA patients had received steroid therapy, cimetidine, or colcicine as a medical treatment for PFAPA symptoms. The mean age at the time of the first antibiotic treatment was 15.4 months (SD 19.1) among the cases and 30.5 months (SD 49.1) among the controls (p < 0.001). Twenty-one of 116 (18%) PFAPA patients and 16/230 (7.0%) controls reported a history of fungal infections (p = 0.001). With regards to subgroups of fungal infections, 14/117 (12%) PFAPA patients and 1/230 (0.4%) controls reported a history of oral thrush (p = 0.003, OR 23.9, 95% CI 3.0 to 188.0).

Hospital visits due to infections during their lifetime were reported by 36/119 (30%) PFAPA patients and 31/230 (13%) controls (p < 0.001). Hospital visits because of other diseases or conditions than infections were as common in the histories of the cases as in the controls (Table 7).

PFAPA cases in the 1st degree relatives were reported by 39/118 (33%) of PFAPA patients. There were no significant differences in self-estimated general health, growth, reported diseases, or the use of antibiotics between the PFAPA patients with or without a positive family history of PFAPA. The health and comorbidities of the classic and incomplete PFAPA patients were also alike (data not shown).
Table 7. Numbers and proportions of 119 PFAPA patients and 230 controls with hospital stays from birth to a mean age of 17 years.

<table>
<thead>
<tr>
<th>Cause of hospital stay</th>
<th>PFAPA patients (n = 119)</th>
<th>Controls (n = 230)</th>
<th>P-value</th>
<th>Proportion difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before TE/TEA (n = 115)</td>
<td>After TE/TEA (n = 116)</td>
<td>All patients who reported hospital visits</td>
<td>All controls who reported hospital visits</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>0 (0.0%)</td>
<td>2 (1.7%)</td>
<td>2 (1.7%)</td>
<td>7 (3.0%)</td>
</tr>
<tr>
<td>Allergy/atopy</td>
<td>5 (4.3%)</td>
<td>4 (3.4%)</td>
<td>9 (7.6%)</td>
<td>15 (6.5%)</td>
</tr>
<tr>
<td>Infections</td>
<td>24 (20.9%)</td>
<td>16 (13.8%)</td>
<td>36 (30.3%)</td>
<td>31 (13.5%)</td>
</tr>
<tr>
<td>Otits</td>
<td>17 (14.8%)</td>
<td>6 (5.2%)</td>
<td>21 (17.8%)</td>
<td>11 (4.8%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0 (0%)</td>
<td>2 (1.7%)</td>
<td>2 (1.8%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Superior respiratory infection</td>
<td>2 (1.7%)</td>
<td>1 (0.9%)</td>
<td>3 (2.5%)</td>
<td>4 (1.7%)</td>
</tr>
<tr>
<td>Laryngitis</td>
<td>5 (4.3%)</td>
<td>0 (0%)</td>
<td>5 (4.2%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>0 (0%)</td>
<td>1 (0.9%)</td>
<td>1 (0.8%)</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Gastrointestinal infection</td>
<td>1 (0.9%)</td>
<td>2 (1.7%)</td>
<td>3 (2.5%)</td>
<td>8 (3.5%)</td>
</tr>
<tr>
<td>Cerebral infection1</td>
<td>2 (1.7%)</td>
<td>1 (0.9%)</td>
<td>3 (2.5%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>3 (2.6%)</td>
<td>3 (2.6%)</td>
<td>5 (4.2%)</td>
<td>8 (3.5%)</td>
</tr>
<tr>
<td>Migraine</td>
<td>1 (0.9%)</td>
<td>2 (1.7%)</td>
<td>3 (2.5%)</td>
<td>3 (1.3%)</td>
</tr>
<tr>
<td>Neurological problem</td>
<td>6 (5.2%)</td>
<td>6 (5.2%)</td>
<td>11 (9.2%)</td>
<td>12 (5.2%)</td>
</tr>
<tr>
<td>Physical trauma</td>
<td>2 (1.7%)</td>
<td>5 (4.3%)</td>
<td>7 (5.9%)</td>
<td>14 (6.1%)</td>
</tr>
<tr>
<td>Tumors1</td>
<td>1 (0.9%)</td>
<td>3 (2.6%)</td>
<td>3 (2.5%)</td>
<td>5 (2.2%)</td>
</tr>
<tr>
<td>Operative treatments</td>
<td>42 (35.3%)</td>
<td>70 (30.4%)</td>
<td>70 (30.4%)</td>
<td>70 (30.4%)</td>
</tr>
<tr>
<td>Tympanostomy</td>
<td>14 (11.8%)</td>
<td>1 (4.8%)</td>
<td>11 (4.8%)</td>
<td>11 (4.8%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>6 (5%)</td>
<td>3 (1.3%)</td>
<td>6 (5%)</td>
<td>3 (1.3%)</td>
</tr>
<tr>
<td>Tumors2</td>
<td>1 (0.8%)</td>
<td>4 (1.7%)</td>
<td>1 (0.8%)</td>
<td>4 (1.7%)</td>
</tr>
<tr>
<td>Congenital anomaly</td>
<td>1 (0.8%)</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
</tr>
</tbody>
</table>

1 CNS central nervous system, 2 No malignant tumors were reported
5.3.1 Microbes of the PFAPA and control tonsils (studies III and IV)

**Bacteria**

In bacterial cultures, 68% (21/31) of the removed PFAPA tonsils and 83% (20/24) of the control tonsils yielded at least one species of pathogenic bacteria (difference 16%, 95% CI -8 to 37%, p = 0.15). The most common finding in both groups was *Haemophilus influenzae* (*H. influenzae*). *Streptococcus pneumoniae* (*S. pneumoniae*) was found more often in the PFAPA tonsils (23%) than in the controls (4%), but the controls were more often tested positive for *Staphylococcus aureus* (*S. aureus*) than the PFAPA cases (38% vs. 10%, Table 8). After adjusting the analyses for age and sex, only the difference in *S. aureus* (OR 0.15, 95% CI 0.03 to 0.76), between the PFAPA group and the controls remained statistically significant (Table 8). *S. pneumoniae* and *S. aureus* seemed to emerge mostly in different patients, and only one control patient tested positive for both. This was also true for *C. albicans* and *S. aureus* (data not shown).

When characterizing the overall microbiome of tonsillar tissue with next-generation sequencing technology, there were 10 phyla and 123 genera in the samples. The majority (99%) of the sequences belonged to the most abundant four phyla: Bacteriodetes (43.1%), Firmicutes (27%) Fusobacteria (24%), and Actinobacteria (5.1%). The most abundant genera among the 123 were *Prevotella* (29.8%), *Fusobacterium* (22.3%), *Parvimonas* (6.8%), *Streptococcus* (6.0%), *Peptostreptococcus* (3.9%), and *Kingella* (2%).

Some statistically significant differences in the mean relative abundances of phylum, genera, and species levels between PFAPA and control tonsil were found. At the phylum level, Cyanobacteria were more abundant in PFAPA cases (0.2, controls 0.02, p 0.01), but at the genera level, Aerococcaceae, Gemallaceae, Granulicatella, Prevotella, and *Streptococcus* were more abundant in controls as well as species like *Prevotella melaninogenica*, *Prevotella nanceiensis*, and *Selenomonas noxia* (Table 9). *Staphylococci* were found in almost all samples in both groups, but the relative abundance was three times higher in the controls than in the cases (p = 0.01).
### Table 8. Microbiological findings in the tonsils of 31 PFAPA patients and 24 controls (microbe cultures, virus PCR, electron microscope).

<table>
<thead>
<tr>
<th>Group</th>
<th>Finding</th>
<th>PFAPA, n= 31</th>
<th>Controls, n= 24</th>
<th>Difference (CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biofilm</strong></td>
<td>Biofilm (+/-)</td>
<td>17 (55%)</td>
<td>5 (24%)</td>
<td>31.0% (3.5 to 53.4%) 0.03</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>Haemophilus influenzae</td>
<td>12 (39%)</td>
<td>9 (38%)</td>
<td>1.2% (-24.6 to 26.2%) 0.99</td>
</tr>
<tr>
<td></td>
<td>Streptococcus pneumoniae</td>
<td>7 (23%)</td>
<td>1 (4%)</td>
<td>18.4% (-0.7 to 36.7%) 0.07</td>
</tr>
<tr>
<td></td>
<td>Fusobacteriae</td>
<td>6 (19%)</td>
<td>2 (8%)</td>
<td>11% (9.4 to 29.9%) 0.28</td>
</tr>
<tr>
<td></td>
<td>Prevotella</td>
<td>4 (13%)</td>
<td>1 (4%)</td>
<td>8.7% (-9.2 to 25.7%) 0.22</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>3 (10%)</td>
<td>9 (38%)</td>
<td>-28.8% (-49.6 to -5.8%) 0.01</td>
</tr>
<tr>
<td></td>
<td>Streptococcus, group G</td>
<td>3 (10%)</td>
<td>4 (17%)</td>
<td>-7.0% (-28.0 to 11.6%) 0.45</td>
</tr>
<tr>
<td></td>
<td>Streptococcus, group C</td>
<td>2 (7%)</td>
<td>1 (4%)</td>
<td>2.2% (-14.9 to 17.5%) 0.99</td>
</tr>
<tr>
<td></td>
<td>Streptococcus, group A</td>
<td>1 (3%)</td>
<td>2 (8%)</td>
<td>-5% (-23.3 to 9.3%) 0.32</td>
</tr>
<tr>
<td></td>
<td>Mycobacteria</td>
<td>1 (3%)</td>
<td>0</td>
<td>3% (-11.0 to 16.4 %) 0.999</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td>C. albicans</td>
<td>5 (16%)</td>
<td>0 2</td>
<td>16.1% (1.1 to 32.8%) 0.003</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>Human herpesvirus 6</td>
<td>16 (53%)</td>
<td>14 (58%)</td>
<td>-5.0% (-30.5 to 21.4%) 0.60</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
<td>14 (47%)</td>
<td>7 (29%)</td>
<td>17.5% (-9.0 to 41.2%) 0.18</td>
</tr>
<tr>
<td></td>
<td>Epstein-Barr virus</td>
<td>8 (27%)</td>
<td>9 (38%)</td>
<td>-10.8% (-35.4 to 14.0%) 0.40</td>
</tr>
<tr>
<td></td>
<td>Enteroviruses</td>
<td>4 (13%)</td>
<td>5 (21%)</td>
<td>-7.9% (-30.0 to 12.3%) 0.32</td>
</tr>
<tr>
<td></td>
<td>Varicella zoster virus</td>
<td>5 (21%)</td>
<td>0 2</td>
<td>-20.8% (-40.7 to -8.0%) 0.007</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex virus 1</td>
<td>1 (4.2%)</td>
<td>1 (4.2%)</td>
<td>-4.2% (-20.5 to 7.7%) 0.22</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex virus 2</td>
<td>1 (4.2%)</td>
<td>2 (8%)</td>
<td>-4.2% (-20.5 to 7.7%) 0.22</td>
</tr>
<tr>
<td></td>
<td>Influenza A virus</td>
<td>1 (4%)</td>
<td>0 2</td>
<td>-4.2% (-20.5 to 7.7%) 0.22</td>
</tr>
</tbody>
</table>

1. Biofilm on the surface of the tonsils was evaluated with scanning electron microscopy. 2. one missing sample. 3. three missing samples. 4. No cytomegal-, influenza B, metapneumo-, parainfluenza 1–3, respiratory syncytial or rhinoviruses were found in PFAPA or control tonsil samples.
Table 9. Mean relative abundance as a percentage (SD) of the bacterial genera or species in the tonsils of 30 PFAPA patients and 24 controls. Only the bacteria with statistically significant differences between the groups are presented.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>PFAPA (n = 30)</th>
<th>Controls (n = 24)</th>
<th>P-value for the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phylum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.2 (1.0)</td>
<td>0.02 (0.07)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Genera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerococcaceae</td>
<td>0.06 (0.1)</td>
<td>0.2 (0.4)</td>
<td>0.048</td>
</tr>
<tr>
<td>Dialister</td>
<td>0.1 (0.3)</td>
<td>0.1 (0.2)</td>
<td>0.012</td>
</tr>
<tr>
<td>Genellaceae</td>
<td>0.5 (0.5)</td>
<td>0.7 (0.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Granulicatella</td>
<td>0.1 (0.2)</td>
<td>0.6 (1.7)</td>
<td>0.003¹</td>
</tr>
<tr>
<td>Prevotella</td>
<td>3.9 (11.2)</td>
<td>4.0 (4.7)</td>
<td>0.017</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>3.7 (4.0)</td>
<td>9.6 (10.3)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevotella melaninogenica</td>
<td>2.2 (2.2)</td>
<td>3.3 (2.1)</td>
<td>0.045</td>
</tr>
<tr>
<td>Prevotella nanceiencis</td>
<td>1.1 (4.3)</td>
<td>2.2 (3.4)</td>
<td>0.001¹</td>
</tr>
<tr>
<td>Selemonias noxia</td>
<td>0.04 (0.1)</td>
<td>0.02 (0.05)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

¹ Statistically significant after Bonferroni correction

**Viruses**

At least one virus was found in either the viral culture or the PCR of 74% (23/31) of the PFAPA tonsils and 83% of the controls (20/24, difference 9%, 95% CI -14 to 30, p = 0.36). The control samples tested positive for VZV and HSV more often than the PFAPA samples (Table 8). After adjusting the analyses for age and sex, only the difference in VZV (OR 0.09, 95% CI 0 to 0.71) between the PFAPA group and the controls remained statistically significant.

**Fungi**

In the yeast culture, the tonsillar tissue of the PFAPA patients yielded *C. albicans* more often than did the controls (16% vs. 0%, p = 0.003, Table 8). No other fungal organisms were detected. In the mycobacterial culture, one tonsil sample in the PFAPA group tested positive for *Mycobacterium bohemicum*.  

55
Biofilm formation

In the SEM of the tonsil biopsies biofilm was found on the surface of 55% of the PFAPA tonsils, but on only 24% of the controls (p = 0.03). *S. pneumoniae* was more commonly cultured in the biofilm-positive (6/22, 27%) than in the biofilm-negative tonsils (2/30, 7%, p = 0.04). The expression of other microbes showed no association with biofilm formation. After adjusting the analyses for age and sex, the differences in biofilm formation (OR 4.24, 95% CI 1.1 to 15.8) between the PFAPA group and the controls remained statistically significant (Table 8).

**Histology**

The PFAPA cases and controls showed no differences in their routine histology findings. All the tonsils showed histological features consistent with reactive follicular lymphatic tissue or follicular hyperplasia. No foci of apparent necrosis or granulomatous reaction were detected. Active inflammation (neutrophils) in 10 (18%) samples was noted but with no predisposition to PFAPA (16% cases and 21% controls, difference 4%, 95% CI -16 to 24%, p = 0.65). Moreover, particles consistent with the morphology of Actinomyces-like organisms were detected in 15 (27%) samples: five PFAPA cases and 10 controls (16% vs. 42%, difference 25%, 95% CI 2 to 48%, p = 0.035). However, after adjusting this analysis for age and sex in logistic regression analysis, the difference was no longer statistically significant. Additional immunohistochemical staining for B and T cells (CD20 and CD3) showed expression patterns consistent with reactive lymphatic tissue (data not shown).
6 Discussion

6.1 Outcome after TE

TE has been shown to be an effective treatment for PFAPA in randomized trials, (Burton et al., 2014; Garavello et al., 2009; Renko et al., 2007) but evidence on the long-term outcome is sparse. In this nine-year follow up, 96% of the PFAPA patients achieved prompt and immediate remission after TE/TEA. In the literature, good results have been reported by Licameli et al. (2012), but with a shorter 3.6-year follow-up time.

In current study, only five patients had recurring fevers at the time of the study visit. Three of them expressed periodic fevers fulfilling the PFAPA criteria. All three patients had healed initially after TE/TEA but experienced a relapse after years of a healthy period. Some reports of PFAPA relapses are found in the literature years after TE/TEA, but also among PFAPA patients after spontaneous healing with no history of operative treatments (Adachi et al., 2011; Colotto et al., 2011; Rigante et al., 2017; Vitale et al., 2016).

6.2 Health

This study is the first controlled study on the long-term postoperative health of PFAPA patients. The patients had excellent self-estimated general current health at the time of the study visit approximately nine years after TE/TEA, and it did not differ from that of matched controls. The incidences of autoimmune and other chronic disease diagnoses were similar in the PFAPA patients and controls, except for pollen allergies, which were less common in the PFAPA cases than in the controls. A long-term prognosis of PFAPA syndrome after TE/TEA seemed to be good even though PFAPA syndrome is considered an autoinflammatory disorder like HIDS, TRAPS, and MEF, which often have a permanent and progressive outcome associated with remarkable co-morbidities after childhood (Cattalini et al., 2016). No differences either in the use of regular medications or operative treatments other than tympanostomy tube insertion were found between the groups.

In the only published cohort study on other comorbidities of PFAPA than infections, 12% of PFAPA patients had symptoms of allergic rhinitis and 8% food or drug allergies at the age of 20 years (Wurster et al., 2011b). In this controlled study, 18% of PFAPA patients and 29% of the controls had a diagnosed allergy, but
when adjusted, the only statistically significant difference was pollen allergies being more common in the controls. In the medical histories of the PFAPA patients, infections and the use of antibiotics were more prevalent among patients than in controls. In the only previous controlled study, no difference was found in the occurrence of infections, recurrent otitis media, and tympanostomy tube insertion between PFAPA patients and controls (Manthiram et al., 2016), but notably, the frequent usage of antibiotics has been reported earlier in other PFAPA cohorts (Førsvoll et al., 2013; Wurster et al., 2011b).

It is problematic to study the history of infections among PFAPA patients. The differential diagnosis of PFAPA is difficult in young children as the clinical status of the fever flares may easily be confused with common viral or bacterial infections, and compared to those infections, PFAPA syndrome is rare. According to the data from the questionnaires of current study, 21% of the PFAPA patients had suffered from infections leading to hospitalizations before the TE/TEA, which was made at the mean age of 4.4 years. After the operation 14% of the patients reported infections leading to hospitalization. Instead, 14% of the controls reported a history of infections leading to hospitalizations throughout their whole lifetime, including also the first years of life. Though, it may be possible that PFAPA patients are more prone to infections than healthy controls, as well as before and after the operative treatment. The incidence of other fungal infections was similar among the cases and controls, but oral thrush was significantly more common among the cases than controls; candida was found also significantly more frequently in the PFAPA. Higher use of antibiotics in the cases may have promoted the occurrence of oral candidiasis.

6.3 Growth

This study is the first controlled publication concerning the growth of PFAPA patients. The growth of the patients has been mentioned to be normal in several publications (Marshall et al., 1987; Marshall et al., 1989; Padeh et al., 1999; Thomas et al., 1999; Wurster et al., 2011b). Normal growth was stated to be a part of the classic Thomas’ diagnostic criteria for PFAPA, and it has been taken for granted, but not studied at all. In this controlled study, the growth of PFAPA patients did not differ from that of the controls. The ethnically homogeneous study population made the use of Finnish growth references for children and adolescents possible in our study. In addition, the intense monitoring of growth of all children
in child health clinics in Finland made the growth-related data collected by the questionnaires reliable.

6.4 Familial cases

In current study, 33% of PFAPA patients reported PFAPA cases in the family. These results are in line with the literature in which the rate of the reported history of familial PFAPA cases of the patients varies from 6% to 45% (Cochard et al., 2010; Førsvoll et al., 2013; Thomas et al., 1999; Wurster et al., 2011b).

In a long-term follow-up study of a PFAPA cohort of Wurster et al., patients with persistent PFAPA were more likely to have a positive family history of the syndrome (44%) than patients with resolved symptoms (4%) (Wurster et al., 2011b), but in current study, the outcome of patients was equally good despite a positive or negative history of familial PFAPA cases.

6.5 Diagnostics

As the etiology of the PFAPA syndrome was and still is unknown, the diagnostic criteria are based on the combination of the clinical features of the syndrome. In the first study population described in 1987, most of the patients were under five at the onset of the fevers, but some were older. These patients also had many heterogeneous additional symptoms (Marshall et al., 1987). Since there was no evidence-based treatment for PFAPA syndrome at the time, it was reasonable to limit the diagnostic criteria to the most common or distinctive features. Although the knowledge of the syndrome has increased, the most commonly used diagnostic criteria are still the initial and classical Thomas’ criteria (Thomas et al., 1999). Modified diagnostic criteria of PFAPA have adopted for use in many clinics, but also criticized in the literature (Hofer, 2008; Manthiram et al., 2017b). As other autoinflammatory syndromes like HIDS, TRAPS, and MEF causing a differential diagnostic challenge in many countries are very rare in the Finnish population, (Korppi et al., 2003) there is clear benefit to investigate diagnostic criteria in Finland and study the profile and outcome of the syndrome between the cases diagnosed with the classic Thomas’ criteria and those with incomplete clinical picture.

Because almost all the PFAPA patients are treated in Oulu University Hospital by TE/TEA, and while it is the main unit treating periodic fever syndromes of the children at the district, we found the most of PFAPA syndrome cases of the area.
The modified PFAPA criteria used in this study (Table 10) found 132 PFAPA patients, of which 119 participated the study. Of those 119 patients, 58 also met the classic Thomas’ criteria, 50 had an incomplete syndrome, and 11 were not possible to classify to these groups due to a lack of information concerning pharyngeal symptoms or the onset age of fevers. In Hofer et al.’s (2014) study, patients who were older at the onset of the syndrome had a more severe disease profile, but no differences between the groups in this study were found. According to our study the fever profile, the health, and the effectiveness of TE did not differ between the group of patients fulfilling the Thomas’ classical PFAPA criteria and those fulfilling the criteria of this study, referring to the same etiological entity. Due to the findings of current work the new diagnostic criteria of PFAPA syndrome, Oulu criteria, are proposed. (Table 10).

Table 10. Oulu criteria for PFAPA syndrome

<table>
<thead>
<tr>
<th>Class</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Regular, periodic fever episodes</td>
</tr>
<tr>
<td></td>
<td>- History of ≥ 5 regular periods</td>
</tr>
<tr>
<td>II</td>
<td>Asymptomatic interval between episodes</td>
</tr>
<tr>
<td>III</td>
<td>Evaluation of the risk for cyclic neutropenia as well as for genetic periodic fevers and diagnostics when necessary</td>
</tr>
<tr>
<td>IV</td>
<td>No other explanation (e.g., respiratory or urinary tract infection) for fever episodes</td>
</tr>
<tr>
<td>V</td>
<td>Normal growth and development</td>
</tr>
</tbody>
</table>

The strengths of studies I and II are the long-term follow-up of a large cohort of PFAPA patients with the study visit and clinical examination. The results concerning the health, growth, and co-morbidities of PFAPA patients are novel, and the large cohort and controlled design strengthens the findings. Still most of the retrospective follow-up data are based on medical records and questionnaires and prone to errors in documentation. The clinical examination of a small child is difficult; thus, it is possible that pharyngeal clinical findings may have gone unnoticed during the fever flares or just not documented by the physician. Recall bias is inevitable, but in order to avoid it the history of diagnosed diseases was asked with multiple questions. The participants were also asked about the reasons for their hospital stays, which might be more memorable to the patients and family members. One of the weaknesses of our study is that the study population consisted only PFAPA patients treated by TE/TEA, not PFAPA patients with a milder disease and spontaneous healing. As such, the results may not apply to all PFAPA patients. In addition, the selection bias of the controls is possible, as controls who actively
responded to our questionnaire may not represent the general population. Furthermore, the timing of the interviews of the controls might have influenced the results, even when adjusted in the analyses.

**6.6 Microbiological and histological findings**

The main features to grasp in solving the mystery of PFAPA syndrome are the clockwork periodicity of the symptoms and the strong effectiveness of TE/TEA in the eradication of the syndrome (Burton et al., 2014; Garavello et al., 2009; Renko et al., 2007; Thomas et al., 1999). The tonsils and pharynx play a crucial role in PFAPA syndrome. To understand more of the mechanisms behind the syndrome, microbiological and histological factors of palatine tonsil samples removed from PFAPA patients and controls were detected in this prospective setting. Marked differences were found in the microbiology of tonsils between PFAPA patients and the controls studied with conventional methods but also with next-generation sequencing technology.

In the histology of the tonsils, no differences were found between the groups. Most of the tonsils expressed the features of reactive follicular lymphatic hyperplasia, reflecting chronic tonsillitis, as previously described in literature among PFAPA patients (Peridis et al., 2010b).

Biofilm formation was more common in PFAPA patients than in controls. Biofilm construction is a survival and defense strategy of microbes against the host’s immune system and antimicrobials. Biofilms are complicated organized constructions, which make antimicrobials ineffective against biofilms, and infections caused by biofilms usually require the removal of the infection focus. This aspect is interesting in PFAPA syndrome, which cannot be cured by antimicrobials, but TE/TEA effectively terminates the symptoms of the syndrome. In this study, more biofilm formation was found in the tonsils of PFAPA patients than in those of the controls, who suffered mainly from tonsillar hypertrophy, although rich biofilm formations have been found in hypertrophied tonsils or chronic tonsillitis (Al-Mazrou & Al-Khatat, 2008; Chole & Faddis, 2003; Diaz et al., 2011b; Kania et al., 2007; Torretta et al., 2013). Biofilms’ life form is cyclic, including four phases: adherence, sessile growth, colonization, and dispersal phases, (Kumar et al., 2017) which is also interesting, as PFAPA has periodic symptom features, although the cycles and activity of biofilms in the pharyngeal area are not well known. The approach to visualizing biofilms in current study was
morphologic without further microbial description of the biofilm types or more specific microbe composition.

Yeasts have been detected from tonsils in children with tonsillar diseases. In a Finnish study yeasts were found from the tonsils of 10 out of 33 patients suffering from tonsillar hyperplasia, recurrent or chronic tonsillitis (Suvilehto et al., 2006). In the study III C. albicans was more common in the tonsil cultures of PFAPA patients than in those of controls, who suffered from tonsillar hyperplasia and the difference was statistically significant. Interestingly, also oral thrush was reported significantly more often among the cases than the controls in the follow-up study II. C. albicans, especially in a hyphal form, has previously been reported to be able to activate inflammasomes and cause pyroptosis and inflammation by triggering the production of pro-inflammatory cytokine IL-1β (Krysan et al., 2014). As the altered activation of inflammasome may be crucial in the pathogenesis of PFAPA syndrome, (Brown et al., 2011; Dytrych et al., 2015) C. albicans might be a potential candidate for further studies.

Pneumococci was found more commonly in the tonsils removed from PFAPA patients than in those removed from the controls; in contrast, samples from PFAPA patients yielded S. aureus less often than that from the controls. As tonsils are rich with microbes and single microbes are not always easily detected with conventional methods, we analyzed the bacterial microbiome of the PFAPA and control tonsils using Ion Torrent high throughput sequencing technology. Typical nasobacterial pathogens like fusobacteria, Prevotella, Tannerella, Porphyromonas, and Parvimonas were found both in patients and controls, but significant differences were also found in the presence and in the abundance of many bacteria. Cyanobacteria were more common among PFAPA patients with higher relative abundance compared with that of the controls. Although streptococci were found in all samples in patients and controls, the mean relative abundance was lower in PFAPA tonsils. In the histological and microbiological studies, a single pathogen possibly causing the syndrome was not found, but clear differences in the microbiome of the tonsils between patients and controls was shown. The syndrome might make the environmental circumstances advantageous to some microbes in the tonsils and disadvantageous to others. Thus, the differences in the tonsillar microbiota could reflect secondary changes in the tonsillar mucosa.

Respiratory viruses are common among young children. Respiratory viruses can be detected in their nasopharynx - either with or without symptoms - nearly half of the year (Byington et al., 2015). Viruses are common also in tonsils. Respiratory viruses are found from 70–75% tonsil tissue samples of asymptomatic
children suffering from tonsillar diseases. The most common viruses detected from the tonsils removed from children suffering from tonsillar hyperplasia are adenovirus, Epstein-Barr virus, human enterovirus, human rhinovirus, human bocavirus and human metapneumovirus (Suvilehto et al., 2006; Faden et al., 2016; Proenca-Modena et al., 2012; Proenca-Modena et al., 2014). In the current study VZV was more often yielded from control tonsils than that of the PFAPA patients. Other yielded or PCR detected viruses did not show significant differences between the groups. Human herpesvirus 6, adenovirus and Epstein-Barr virus were the most common viruses in both groups. As many viruses are difficult to detect by cultures and PCR kits used in this study covered only limited number of viruses, in further studies virus detection with modern methods could reveal significant pathogens behind the syndrome.

The strength of this microbiological study is the broad microbiological analysis of PFAPA and control tonsils. The high carriage rates of respiratory pathogens in this study are in line with previous findings on bacteria and viruses (Proenca-Modena et al., 2012; van den Bergh et al., 2012). The most important limitation of the study was that the control patients suffered from tonsil hypertrophy, in which tonsils microbiome is also variable and rich (Proenca-Modena et al., 2014; Faden et al., 2016; Wang et al., 2017). However, getting tonsil tissue samples from healthy pediatric patients is difficult. Along the Danish study of the bacterial microbiota of the tonsillar crypts, the microbiome in tonsil hyperplasia was closer to that of asymptomatic patients than patients suffering from recurrent tonsillitis, thus control samples from tonsil hyperplasia patients may bring as little bias as possible to the results (Jensen et al., 2013). Another limitation of the study was that the controls were slightly older than the PFAPA patients, possibly influencing the colonization of the nasopharynx and the occurrence of PFAPA. Therefore, all the analyses were adjusted by age with logistic regression modeling. Antimicrobial medications prior to TE also influence the microbes of the tonsils. Although data on the usage of antimicrobials one year before TE was convincing, frequent antimicrobial treatments of PFAPA patients may have had some influence on the microbe findings. The tonsil samples of the patients were taken between the flares, which may not be the optimal time to detect the possible microbe triggering the fever flare, but pediatric TE/TEA in the beginning or at the time of fever is ethically controversial. In further settings the detection of viruses and fungi of tonsils should be analyzed with next generation sequencing methods.
7 Conclusion

The effect of TE/TEA or the clinical profile of PFAPA syndrome did not differ between the patients diagnosed with the classic Thomas’ criteria and those diagnosed by modified diagnostic criteria, Oulu criteria, used in this study. The proposed new criteria allow the later onset of the syndrome and/or absence of aphthous stomatitis, pharyngitis, or adenitis.

In this nine-year follow-up study, the effect of TE/TEA in the treatment of PFAPA syndrome was unwavering and the long-term health, growth, and frequency of autoimmune or other chronic diseases of the patients were comparable to healthy controls. The microbes on the tonsils of PFAPA patients in this controlled study differed markedly. In particular, pneumococci, abundant biofilm formation on the surfaces of the removed tonsils and C. albicans were found significantly more often in tonsils of PFAPA patients than controls. Statistically significant differences were found in the proportions and abundance of some genera between the patients and controls. Histological differences were not found between the groups. It is possible that microbes have a role in the regulation of inflammatory processes triggering the symptoms of PFAPA.
References:


Original publications


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1461. Pasanen, Anu (2018) Genetic susceptibility to childhood bronchiolitis

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1465. Isojärvi, Henri (2018) Association of glucose metabolism, physical activity and fitness with peripheral nervous system function in overweight people


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1472. Mäihä, Mari (2018) Genetic background and antenatal risk factors of bronchopulmonary dysplasia

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ETIOLOGY AND OUTCOME OF PFAPA (PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS AND ADENITIS) SYNDROME AMONG PATIENTS OPERATED WITH TONSILLECTOMY IN CHILDHOOD

Ulla Lantto