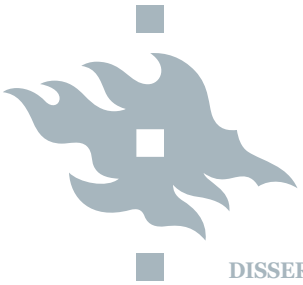

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Oral Manifestations in Crohn´s Disease and Orofacial Granulomatosis

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ORAL MANIFESTATIONS IN CROHN´S DISEASE AND OROFACIAL GRANULOMATOSIS

Anu Haaramo

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of
the University of Helsinki, for public examination in the Richard Faltin –lecture hall, Surgical Hospital, Kasarmikatu 11-13, on 10th of May 2019, at 12 noon.

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To My Family

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Haaramo A, Alapulli H, Aine L, Saarnisto U, Tuokkola J, Ruuska T, Sipponen T, Pitkäranta A, Kolho KL. Detailed Follow-up Study of Pediatric Orofacial Granulomatosis Patients. J Pediatr Gastroenterol Nutr. 2017 Oct; 65(4): 388-393.

II Paakkanen R, Haaramo A, Kolho KL, Ruuska T, Lokki ML. HLA-B*44 May Be a Marker for Orofacial Granulomatosis. J Pediatr Gastroenterol Nutr. 2016 Nov; 63(5):e121-e122.

III Haaramo A, Alapulli H, Aine L, Tuokkola J, Saarnisto U, Roine RP, Pitkäranta A, Kolho KL. Oral and otorhinolaryngological findings in adults who were diagnosed with pediatric onset Crohn´s disease: a controlled study. J Clin Gastroenterol. 2018 Jun 16 doi: 10.1097/MCG.0000000000001074. [Epub ahead of print]

IV Haaramo A, Kolho KL, Pitkäranta A, Kanerva M. A 30-year Follow-up Study of Patients with Melkersson-Rosenthal syndrome shows an Association to Inflammatory Bowel Disease. *Accepted for publication in Annals of Medicine*.

The publications are referred to in the text by their roman numerals and can be found at the end of this thesis. Publications are reprinted with the permission of the copyright holders.

ABBREVIATIONS

ASCAb	Anti-Saccharomyces cerevisia antibody
ASCAbA	Anti-Saccharomyces cerevisia antibody A
ASCAbG	Anti-Saccharomyces cerevisia antibody G
CD	Crohn´s disease
CG	Cheilitis granulomatosa
CRP	C-reactive protein
DMFT	The decayed, missing, and filled teeth index
EEN	Exclusive Enteral Nutrition
ESR	Erythrocyte sedimentation rate
HLA	Human leukocyte antigen
HRQoL	Health-related quality of life
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
IL	Interleukin
IL-2	Interleukin-2
MRS	Melkersson Rosenthal syndrome
NOD2	Nucleotide binding oligomerization domain containing protein 2
OFG	Orofacial granulomatosis
TNF	Tumor necrosis factor
Th1	T helper 1 cell
UC	Ulcerative colitis
VAS	Visual analog scale

ABSTRACT

Background: Crohn´s disease (CD) is a chronic inflammatory disease that can affect any part of the digestive tract, including the mouth. The reported prevalence of oral manifestations in Crohn´s disease varies widely and ranges from 0.5% to 80%. Oral manifestations may coincide with the intestinal symptoms or they can precede them.

Orofacial granulomatosis (OFG) is a rare chronic inflammation presenting in the oral cavity and around the mouth without intestinal involvement. Histology shows non-caseating granulomas. Melkersson-Rosenthal syndrome (MRS) is considered to be one manifestation of OFG, where granulomatous inflammation and oedema is accompanied by recurrent facial palsy and fissured tongue. Oral lesions in CD, OFG, and MRS cannot be clinically or histologically distinguished.

Patients with OFG may develop a symptomatic gut disease and it is debated whether OFG is an oral manifestation of CD, or a separate inflammatory disorder. In addition, it is unknown whether patients with MRS are prone to develop CD in follow-up.

The etiology of OFG is unknown. Various etiological mechanisms have been suggested. Human leucocyte antigen (HLA) genes affect inflammatory and immune responses and certain HLA types are associated with distinct inflammatory, infectious and autoimmune conditions. HLAs are also involved in the pathogenesis of CD. The role of HLA in OFG is not well-established.

Methods: First, patients with pediatric-onset OFG were examined. A cohort of 29 patients with pediatric-onset OFG were described in detail, including otorhinolaryngological and dental examinations, nutrition from food records, and laboratory tests. Orofacial findings were photographed and recorded using a structural schema. The findings were compared between patients with OFG only and OFG with CD. Possible factors predicting bowel disease in orofacial patients were of special interest.

Next, a case-control study was performed on 24 patients with pediatric-onset CD who had reached adulthood and 22 matched healthy controls. The presense of oral and otorhinolaryngological manifestations in patients with pediatric-onset CD in adulthood were investigated. Orofacial findings were evaluated and recorded as described in the first study. Questionnaires were used to evaluate patients´ general health and health-related quality of life (HRQoL). Nutrition was evaluated from food records.

Last, a phone-interview was performed with 27 patients suffering triad or oligosymptomatic MRS or its monosymptomatic form cheilitis granulomatosa

(CG). The health outcomes of these patients in long-term follow-up with a special emphasis on intestinal symptoms, were of interest. The aim was to evaluate the relationship between OFG, MRS, and CD.

Blood samples were collected from patients with OFG, patients with CD and controls to investigate inflammatory markers, haemoglobin, and anti-Saccharomyces cerevisiae antibodies A (ASCAbA) and G (ASCAbG). In patients with OFG, the HLA-haplotypes were also examined. HLA-haplotypes of patients with OFG were constructed computationally and the results were compared to control cohorts of pediatric patients with CD and general population.
Stool samples were collected from all patient groups to evaluate faecal calprotectin, a surrogate marker for intestinal inflammation.

Results: Of the 29 patients with pediatric-onset OFG, 21 (72%) developed CD during the time between diagnosis and this study (median 3.1 years). ASCAbA levels were higher in patients who developed a symptomatic gut disease compared to those with OFG only. Patients with elevated ASCAbA also had more oral findings compared to patients with normal levels of ASCAbA ($p=0.0311$). In addition to faecal calprotectin, ASCAbA may serve as a factor predicting underlying or developing CD in patients with OFG, but further studies are needed.

Of the 27 patients with MRS or CG, only one (3.7%) CG patient developed CD during follow-up of a median 30 years. However, two MRS patients developed ulcerative colitis, and thus in total 11.1% had a diagnosis of inflammatory bowel disease.

The patients with OFG only and OFG with CD did not differ significantly in the HLA-allele or HLA-haplotype frequency. However, HLA-B*44 was more common in patients with OFG with CD than in the control group of patients with pediatric CD. Compared to the general population, the frequency of HLA-B*44 was twice as high in patients with OFG and CD, but the difference was statistically not significant. None of the other HLA-alleles or haplotypes linked with OFG.

At the time of the study, almost all patients with OFG had oral lesions, but were mostly only slightly symptomatic. Oral mucosa was the most common site for orofacial findings, affecting 90% of patients with OFG. In patients with pediatric-onset CD, oral lesions did not seem to increase during the course of the gut disease and they seemed to be inactive when the gut disease was under good therapeutic control. Interestingly, oral lesions were more likely to be seen in patients with CD with a history of perianal abscesses. Over half of the patients with MRS or CG reported oral manifestations at some point during the disease course. Angular cheilitis was common in all patient groups.

None of the nutritional factors and none of the dental findings correlated to oral findings in patients with OFG or in patients with pediatric-onset CD in adulthood.

Otorhinolaryngological findings were minor in both patients with pediatric OFG and patients with pediatric-onset CD in adulthood.

Conclusion: Prognosis for OFG is good. Symptoms tend to diminish during the course of the disease and no severe disease exacerbations were seen. In patients with CD the oral symptoms seem to be inactive when the gut disease is under good therapeutic control. Pediatric CD confers only a modest risk of oral lesions at adult age.

OFG and CD do not seem to increase otorhinolaryngological comorbidity.

One possible factor was found that was associated with gut disease in patients with OFG. Elevated ASCAbA may serve as a factor predicting the underlying or developing CD in patients with OFG. On the other hand, patients with CD and a history of perianal abscessing disease seem to be prone to oral manifestations of CD.

MRS has a recurrent and chronic disease course. Oral findings are relatively common in patients with MRS. This study´s findings suggest a link between MRS and IBD.

SUMMARY IN FINNISH

Crohnin tauti on krooninen tulehduksellinen suolistosairaus, jonka esiintyvyys kasvaa nopeasti länsimaissa. Crohnin tauti voi esiintyä missä tahansa ruoansulatuskanavan osassa, myös suussa. Crohnin taudin suumuutokset kuvattiin ensimmäisen kerran vuonna 1969, mutta suumuutosten käyttäytymistä ja yleisyyttä taudin eri vaiheissa ei edelleen täysin tunneta.

Orofasiaalisella granulomatoosilla tarkoitetaan kasvojen ja suun alueelle rajoittunutta pitkäaikaista tulehduksellista tilaa, jolle tyypillistä on kasvojen ja huulien turvotus sekä suun limakalvojen haavaumat. Orofasiaalisen granulomatoosin etiologiaa ei täysin tunneta. Ratkaisua on etsitty viivästyneestä allergisesta reaktiosta, infektioista, immunologiasta ja perintotekijöistä. Todennäköisesti etiologia on monitekijäinen.

Orofasiaalista granulomatoosia ja Crohnin taudin suumuutoksia ei voida erottaa toisistaan kliinisesti tai histologisesti. Muutosten histologia osoittaa nonkaseoottista granulomatoottista tulehdusta. Histologialtaan vastaavia suumuutoksia voi esiintyä myös sarkoidoosissa ja tuberkuloosissa. Orofasiaalisen granulomatoosin taustalla voikin piillä systeemisairaus, kuten Crohnin tauti tai sarkoidoosi, mutta usein tila tulkitaan idiopaattiseksi oireyhtymäksi. Osalle orofasiaalista granulomatoosia sairastavista kehittyy oireinen Crohnin tauti. Melkersson-Rosenthalin oireyhtymässä orofasiaaliseen turvotukseen ja granulomatoottiseen tulehdukseen liittyy perifeerinen kasvohalvaus ja uurrekieli. Melkersson-Rosenthalin oireyhtymää on pidetty yhtenä orofasiaalisen granulomatoosin alamuotona. Sitä, liittyykö Melkersson-Rosenthalin oireyhtymään riski sairastua Crohnin tautiin pitkäaikaisseurannassa, ei tiedetä.

Tässä väitöskirjatyössä tutkittiin orofasiaalisen granulomatoosin, Melkersson-Rosenthalin oireyhtymän ja Crohnin taudin yhteyttä. Lähestyimme kasvo- ja suuoireita eri näkökulmista. Ensin tutkittiin orofasiaalista granulomatoosia sairastavat potilaat ja etsittiin tekijöitä, jotka voisivat liittyä suolistosairauden kehittymiseen suuoireisilla potilailla. Seuraavaksi selvitettiin lapsuudessa Crohnin tautiin sairastuneiden jo aikuisiksi varttuneiden potilaiden suuoireita. Crohnin tautia sairastavien potilaiden tuloksia verrattiin terveisiin verrokkeihin, jotka poimittiin Väestörekisteristä. Viimeisenä selvitettiin Melkersson-Rosenthalin oireyhtymää ja sen yksioireista ja yleisempää alamuotoa, cheilitis granulomatosaa sairastavien potilaiden oireilua pitkäaikaisseurannassa.

Orofasiaalista granulomatoosia sairastavien potilaiden suuoireilu ei tutkimuksessa lisääntynyt taudin edetessä. Jopa 72%:lla potilaista todettiin Crohnin tauti ja hieman yllättäen näillä potilailla oli myös enemmän suulöydöksiä verrattuna niihin potilaisiin, joilla oli vain orofasiaalinen granulomatoosi. Koholla olevat IgA-luokan vasta-aineet Anti-saccharomyces cerevisie:lle (ASCAbA) olivat yhteydessä

Crohnin tautiin ja runsaampiin suulöydöksiin verrattuna potilaisiin, joilla ASCAbA ei ollut koholla. Tutkimuksen perusteella ASCAbA voisi olla tekijä, joka osoittaa piilevää tai kehittyvää suolistosairautta suuoireisilla potilailla, mutta jatkotutkimuksia tämän osalta tarvitaan.

HLA-alleelin tai HLA-haplotyyppien osalta potilaat, joilla oli orofasiaalinen granulomatoosi ja potilaat, joilla oli orofasiaalinen granulomatoosi ja Crohnin tauti, eivät eronneet toisistaan tilastollisesti merkitsevästi. HLA-B*44 oli yleisempi niillä potilailla, joilla oli orofasiaalinen granulomatoosi ja Crohnin tauti, kuin niillä potilailla, joilla oli vain Crohnin tauti. Mikään muu HLA-alleeli tai -haplotyyppi ei ollut yhteydessä orofasiaaliseen granulomatoosiin. HLA-B*44 voisi viitata lisääntyneeseen OFG:n riskiin, mutta jatkotutkimuksia tarvitaan.

Crohnin tautia lapsuudesta sairastaneiden potilaiden suulöydökset olivat tutkimuksessa vähäisiä. Suuoireilu ei näytä lisääntyvän suolistosairauden edetessä ja on vähäistä silloin, kun suolistosairaus on hyvässä hoitotasapainossa. Crohnin taudin perianaalinen abskessoiva tautimuoto oli yhteydessä lisääntyneisiin suuoireisiin, mutta muita selviä suuoireisiin liittyviä tekijöitä ei todettu.

Orofasiaalista granulomatoosia tai Crohnin tautia sairastavien potilaiden otorinolaryngologisessa tutkimuksessa ei tullut esille poikkeavaa. Näihin tauteihin ei näytä liittyvän lisääntynyttä sairastavuutta korva-, nenä- ja kurkkutautien osalta.

Crohnin tautiin tiedetään liittyvän lisääntynyt riski hampaiston ongelmille. Tutkimuksessa mikään hammaslöydös ei ollut yhteydessä suumuutosten esiintymiseen potilailla, joilla oli orofasiaalinen granulomatoosi tai Crohnin tauti. Ientulehdus oli potilailla hyvin yleistä.

Potilaiden ravitsemuksen tutkiminen ei tuonut esille selvää suumuutoksiin yhteydessä olevaa tekijää.

Melkersson-Rosenthalin oireyhtymää tai cheilitis granulomatosaa sairastavien potilaiden haastattelututkimuksessa ilmeni, että yli puolella potilaista oli ollut jossain taudin vaiheessa suuoireita. Vain yksi cheilitis granulomatosaa sairastava potilas sairastui Crohnin tautiin 30 vuoden (mediaani) seurannan aikana. Kuitenkin kahdella Melkersson-Rosenthalin oireyhtymää sairastavalla potilaalla todettiin haavainen paksusuolitulehdus seuranta-aikana, joten yhteys Melkersson-Rosenthalin oireyhtymän ja tulehduksellisten suolistosairauksien välillä on mahdollinen.

1 INTRODUCTION

Crohn´s disease (CD) is a chronic inflammatory disease of the gastrointestinal tract with increasing incidence worldwide. The incidence of CD is highest in Europe and North America, ranging from 10 to 30 cases per 100 000 person-years [1]. The incidence of CD in Finland is 9.2 cases per 100 000 inhabitants per year [2].

Terminal ileum and colon are the most common segments of the gastrointestinal tract affected in CD but it can also manifest in the oral cavity. The oral manifestations in CD were first described in 1969 [3]. It has been estimated that 0.5 – 50% of patients with CD have oral manifestations and in children with CD the prevalence is even higher.

Granulomatous inflammation manifesting with facial oedema and facial palsy was first described by Melkersson in 1928 [4]. In 1931, Rosenthal presented a patient with orofacial swelling, facial palsy and lingua plicata, and suggested a connection between these findings [5]. Since 1949, the triad has been known as Melkersson-Rosenthal syndrome (MRS) [6]. Orofacial granulomatosis (OFG) was first introduced in 1985 to describe orofacial swelling and granulomatous inflammation without evidence of systemic disease [7]. The term “OFG” encompasses the previously described entities with granulomatous inflammation in the orofacial area without any underlying systemic condition. However, the connection between OFG and MRS is not completely clear, and there is conciderable overlap in the classification.

It is known to date that patients with OFG can develop a symptomatic gut disease and patients with CD may have oral manifestations. It is debated whether OFG is an oral manifestation of CD, or a distinct inflammatory entity. The possibility of a third entity, namely OFG with gastrointestinal involvement, has been proposed recently. Clinicians are particularly interested in understanding the factors that predict underlying or future gut disease in patients with OFG. In addition, the relationship between MRS and CD is not well established.

Orofacial lesions in both CD and OFG can cause notable discomfort and concern in patients. Due to a growing incidence of IBD, the number of people who suffer from orofacial lesions is growing. These patients are encountered by physicians in many specialities. Pediatricians, dentists, gastroenterologists, and otorhinolaryngologists, as well as general practitioners, should be aware of this still rare entity. Gaining more knowledge of this disease spectrum is the key for better understanding and helping patients.

2 REVIEW OF THE LITERATURE

2.1 CROHN´S DISEASE

Ulcerative colitis (UC) and Crohn´s disease (CD) are chronic inflammatory bowel diseases (IBD). For both diseases, autoimmune type inflammation is typical. UC is restricted to the mucosa of colon, whereas CD can affect any part of the digestive tract. Disturbances in immune responses and altered intestinal microbiota play important roles in the pathology of IBD [8]. A genetic predisposition is also evident, although significant predisposing factors are yet unknown [9]. The incidence of IBDs is growing rapidly in Western countries [1,10]. In Finland, UC is still more common than CD, but in the rest of Europe, CD is notably more common [11-13]. The onset of CD typically occurs in the second to fourth decade of life, but the disease can occur also in childhood. About 25% of the cases are diagnosed during childhood [8].

CD typically affects the terminal ileum and colon but has potential to affect any part of the gastrointestinal tract including the oral cavity [14]. Oral manifestations in CD were first described in 1969, but it remains uncertain regarding how frequently these manifestations occur among patients with CD [15] and how they act during different stages of gut disease. Oral manifestations may coincide with the intestinal symptoms of CD or precede them.

Oral manifestations in CD are typically divided into disease-specific and non-specific lesions. Specific lesions result from the same disease process as CD in the gut, namely granulomas that can be identified by histology. These specific lesions include swelling of the lips, cheeks and gingiva, cobblestone formation of the mucosa, deep linear ulcers, and mucosal tags [16-18]. Lesions may be preceded by painless gingival enlargement [19]. Non-specific lesions are reactive lesions without granulomas [18]. Patients with CD often suffer from aphthous ulceration, angular cheilitis, lip fissuring, and gingivitis, but these are not specific to CD [18]. The most common lesion that has been reported is cobblestoning, which is described by fissured swollen buccal mucosa with corrugation and hyperplasia of the mucosa, followed by linear ulcers [20]. The lips [21] and labial commissure [22] have been reported to be the most frequently affected sites. In reviews on pediatric patients, mucogingivitis [17] and oral ulceration [18] have been reported to be the most common oral lesions.

In the published literature, the reported prevalence of oral manifestation in patients with CD has varied widely, ranging between 0.5% and 50% in adult cohorts [17,23,24] and between 10% and 80% in pediatric cohorts [18]. The wide variation in prevalence numbers can partly be explained by different age groups

and ethnic groups, experience of the examiner, definition of disease-specific lesions and whether the patient is on treatment for CD at the time or not [17].

2.2 OROFACIAL GRANULOMATOSIS

Orofacial granulomatosis (OFG) is an uncommon chronic condition described by granulomatous inflammation restricted to the orofacial area. The term was first described in 1985 as an idiopathic entity of orofacial swelling and granulomatous inflammation without evidence of systemic disease [25]. Primary symptoms are oedema in the lips and face, and ulceration in the mouth [25]. Histology shows noncaseating granulomas. Oral lesions identical to OFG can be seen in CD, tuberculosis, and sarcoidosis. OFG can be accompanied by a systemic disease like CD or sarcoidosis, but usually it is referred as an idiopathic entity of its own [26]. In Melkersson-Rosenthal syndrome (MRS), orofacial oedema and granulomatous inflammation are accompanied by facial palsy and fissured tongue (lingua plicata) [6,27]. Cheilitis granulomatosa (CG) is considered to be a monosymptomatic form of MRS. It comprises chronic facial or labial swelling due to granulomatous inflammation. The major findings in OFG, MRS, and CG are described in Table 1.

OFG may be used as an umbrella term to encompass all previously described granulomatous entities in the orofacial area [28], including MRS and CG. However, there is conciderable overlap in the nomenclature and precise classification is not always even possible, especially on initial presentation when all clinical features may not be present.

Table 1. *Typical clinical features in orofacial granulomatosis (OFG), Melkersson-Rosenthal syndrome (MRS) and cheilitis granulomatosa (CG).*

Typical clinical features		
OFG	*MRS*	*CG*
facial oedema labial oedema ulceration in the mouth	recurrent orofacial oedema recurrent facial palsy fissured tongue	facial or labial oedema

2.2.1 ETIOLOGY

The etiology of OFG remains largely unknown. Etiological factors have been sought from various directions. Genetic, immunologic, allergic, and infectious mechanisms have been proposed [26,28,29]. Due to various study results the etiology seems most likely multifactorial.

2.2.1.1 Genetic mechanisms

A familial association was suggested after a study of 42 patients with OFG and their 171 relatives found lingua plicata in 23% of the families [30]. It has since been reported that 10% of the normal population can have lingua plicata [31], which weakened the assumption of a familial association.

The human leukocyte antigen (HLA) system is a gene complex encoding the major histocompatibility complex in humans. HLAs are cell-surface proteins responsible for the regulation of the immune system. HLAs are highly polymorphic and have multiple different alleles and therefore fine-tune the adaptive immune system. Certain HLA types are associated to distinct inflammatory, infectious and autoimmune conditions, like type 1 diabetes and celiac disease [32]. HLAs are also involved in the pathogenesis of CD. Interest in the possible connection between OFG and HLA has risen. Whilst two studies failed to show a strong association between HLA and pathogenesis of OFG [33,34], HLA genotypes A3, B7, and DR2 were associated with OFG in a small study of 16 patients with OFG compared with normal population [35]. In another study, HLA-B16 and HLA-CW3 were more common in patients with MRS compared to normal population, but the difference was not statistically significant [33]. Results with small study cohorts show that OFG might link to specific HLA genotypes and haplotypes, but further studies are needed.

2.2.1.2 Allergic mechanisms

Delayed-type hypersensitivity reaction, particularly contact hypersensitivity, to various food substances and food additives has been proposed as etiology of OFG [26,36-38]. Wheat, dairy products, chocolate, eggs, peanuts, cinnamaldehyde, piperitone, cocoa, carvone, carmoisine, sun yellow dye, monosodium glutamate, and benzoic acid, for example, have been reported as causative or contributing agents to OFG [26,28]. Up to 60% of the patients with OFG are atopic, which supports the theory of an allergic background [39-41]. Allergy to tooth-paste and dental materials, such as mercury and amalgam, has also been described in case reports [42-44]. A study evaluating the utility of cutaneous patch testing showed that patients with OFG were more likely to have reactions to food additives,

especially benzoic acid and chocolate, than other disease cohorts or controls [45]. No reactions to cutaneus patch tests for mercury or other metallic salts, indicating reactions to amalgam, were observed in patients with OFG [45].

Delayed-type hypersensitivity reaction appears to play a significant role according to case reports, although the exact antigen inducing the immunological reaction seems to vary in individual patients [26]. Avoidance of causative substances may help some patients to manage the symptoms of OFG [39,46,47], but it is unknown whether these substances are the primary causative agents or exacerbate the existing disease.

2.2.1.3 Infectious mechanisms

The involvement of microbiological agents in the etiology of OFG has been suggested, but the definite role of infections remains unclear. Studies focus mainly on Mycobacterium tuberculosis, Mycobacterium paratuberculosis, Saccharomyces cerevisiae and various types of spirochetes [26]. Studies investigating the role of mycobacteria in the etiology of OFG have comprised small patient groups, and shown controversial results, thus the relationship between mycobacteria and OFG remains unclear [28]. Anti-saccharomyces cerevisiae antibodies (ASCAb) have been associated with CD [48], and one study has reported an association to oral involvement [48,49]. Assessment of anti-saccharomyces cerevisiae antibodies in the serum and saliva of patients with OFG and CD demonstrated that, salivary IgA antibodies was higher in patients with oral disease, and serum IgA antibodies were raised in the groups with gut disease [50]. This suggests that serum IgA antibodies to Saccharomyces cerevisiae may serve as a serological marker for the development of gastrointestinal involvement in patients with OFG [50]. The role of Borrelia burgdorferi in OFG was investigated in a small study, but Borrelia burgdorferi antibodies could not be detected in the serum of the patients [51]. However, in patients with CG and MRS, antibodies against Borrelia burgdorferi were found in up to 82% of the patients [52]. The role of spirochetes remains unclear, although Chinese studies have demonstrated the presence of spirochetes in Chinese patients with CG or MRS [52-54].

2.2.1.4 Immunologic mechanisms

OFG is characterized by non-caseating epithelioid granulomas. Additional features include significant lymphoedema and both diffuse and focal aggregates of lymphocytes, mostly CD4+ T cells [25]. One study compared the T-cell surface receptors present on lymphocytes at the site of the lesion to those of peripheral blood lymphocytes and found no significant difference [55]. This indicates that the accumulation of T cells in OFG is not driven by a specific antigen, but rather a

random influx of inflammatory cells [55]. The idea of random influx of T-cells at the site of inflammation was supported in a later study [56]. Clonal expansion of peripheral T cells has also been reported [57]. Elevation of IL-2 in peripheral blood lymphocytes has been also reported, which suggests that circulatory lymphocytes are activated in OFG [58]. A significant increase of IFN-γ expression in oral lesions of OFG together with increased levels of IL-12 suggest a Th1-mediated immune response in patients with OFG [59]. Increased levels of certain chemokines and chemokine receptors also indicate Th1-mediated immune response with strong resemblance with the inflammatory reaction present in gut lesions of patients with CD [59]. In addition, subepithelial dendritic B-cells have also been found in biopsies from patients with OFG [60].

2.2.2 EPIDEMIOLOGY

The precise prevalence and incidence of OFG is not known. OFG appears to be most prevalent in young adults, although it may occur at any age [28]. OFG seems to affect both sexes equally and is not restricted to any racial group [28]. Nonetheless, it has been suggested that OFG is more prevalent among "Celtic" populations [61]. A recent study from Sweden suggests that there might be geographical differences in the epidemiology of OFG [62]. In the Swedish cohort, there was a clear predominance of males (female/male ratio 1:2.6), whereas the patients in the UK and Ireland have showed a more equal gender distribution [62-64]. In the Swedish cohort, the patients were also younger with a median age of 14 years (range 7-32) compared to those reported from the UK (median 23 years) and Ireland (median 28 years) [62-64].

2.2.3 CLINICAL FEATURES

OFG classically presents with non-tender recurrent labial oedema that may become persistent after recurrences [25]. More recently, it has been understood that OFG has a very variable presentation. It may affect only the lips, which is the most common site, or all facial tissues. Typical is painless and nonpruritic oedema that may be mild and recurrent, but may progress to more severe, persistant oedema causing cosmetic and functional problems. The oedema may be asymmetric, involving one or both lips or only one side of the lip. The swollen lip may feel soft, firm or nodular on palpation, and when the swelling has become chronical, rubbery firm. Labial oedema can be accompanied by lip scaling and vertical fissures of the lips. Oedema can also manifest in other facial tissues. Oral manifestations include angular cheilitis, mucosal ulceration, mucosal tags, swelling of the mucosa, and fissured dorsum of the tongue (lingua plicata) [25]. Generalized swelling of the buccal mucosa leads to a cobblestone-like appearance. Localized mucosal swelling manifests as painless tags in the buccal or labial

mucosa, or in the floor of the mouth where the finding is described as staghorning. Deep chronic linear ulcers in the buccal or labial sulcus can cause significant pain [65]. Gingivitis and gingival overgrowth is also reported.

The most common presentation of OFG is lip swelling and facial swelling, which are reported in more than 90% of the cases [64,66]. Labial swelling was the most common presenting clinical feature at diagnosis found in over 75% of the 49 patients [67].

2.2.4 HISTOLOGY

Characteristic histopathological features include non-caseating, i.e. non-necrotising, granulomas with multinucleate giant cells, dilated lymphatics, and a perivascular lymphocytic infiltrate [7,29,59]. However, nonspecific infiltration of inflammatory cells may be the only histopathological finding in biopsies, as granulomas are not always present [6,68]. OFG and oral lesions in CD cannot be histologically distinguished. A recent study evaluated the histopathology of 22 patients with OFG and found discrete loose epithelioid cell or lymphonodular granulomas as the most specific finding in 73% of the patients. None of the patients in this study developed CD, but the authors agreed that OFG is histopathologically similar to lesions of CD [69]. The perilymphatic disposition of granulomas and the presence of intralymphatic histiocytes or intralymphatic granulomas may explain the dilatation of lymphatic vessels and the development of oedema [69].

2.2.5 DIAGNOSIS

The diagnosis of OFG is clinical and based on patient´s history and typical clinical features. Biopsy of an affected area for hematoxylin and eosin staining may be helpful to find histologic evidence of noncaseating granulomas. However, biopsy is not mandatory for the diagnosis, and can also be negative for granulomas [6,68,70]. To date, additional diagnostic studies are tailored for each individual patient and mostly aim to exclude other conditions characterized by granulomas, including CD, tuberculosis, and sarcoidosis. Additional diagnostic studies may include skin patch or prick testing (to assess allergy), chest radiography (hilar adenopathy found in tuberculosis and sarcoidosis), assessment of angiotensin converting enzyme (increased in sarcoidosis), levels of hemoglobin, folate, iron/ferritin and vitamin B12 (decreased in CD), inflammatory markers (increased in CD), tuberculosis testing (tuberculin skin test or interferon γ release assays) and if gastrointestinal involvement is suspected, endoscopy including biopsies [28,29]. Capsule-endoscopy has its place in investigating the small intestine in case of bowel-related symptoms or when gastrointestinal involvement is suspected.

Childhood onset, laboratory abnormalities, and gastrointestinal symptoms are features that may indicate a need to evaluate for concurrent or future CD [28].

2.2.5.1 Differential diagnosis

Differential diagnosis includes trauma, infection and angioedema, which are the most common reasons for labial edema. However, in these conditions labial swelling is typically transient. Allergic angioedema may manifest as non-pitting oedema of the lips, tongue, pharynx and face. A predispositional factor may be identifiable and patients may have a history of atopy, which however is often true for patients with OFG as well. Differential diagnosis includes other conditions characterized by granuloma formation, including CD, tuberculosis and sarcoidosis. In CD orofacial findings are similar to those of OFG, although oro-cutaneous fistulas and pyostomatitis vegetans are suggestive of CD [28,29,71]. Tuberculosis manifests as localized labial swelling and ulcers, and lesions are often severe [72]. In tuberculosis histology usually contains caseating granulomas, whereas in OFG the granulomas are non-caseating. Risk factors for tuberculosis include HIV, immigration from high-risk regions, alcoholism, intravenous drug use and diabetes mellitus. In chronic sarcoidosis, patients may also have pulmonary, cutaneous, lacrimal, salivary, neurological and skeletal features of sarcoidosis. Granulomatosis with polyangiitis, formerly Wegener´s granulomatosis, can also include oral lesions, although other symptoms dominate [73,74]. Cheilitis glandularis causes labial enlargement with ulcers and intermittent discharge of clear fluid. Histology in cheilitis glandularis shows non-granulomatous inflammation in minor salivary glands of the lip [29,71,75]. Other conditions causing orofacial symptoms that may resemble OFG include rosacea, acne vulgaris, contact dermatitis, exfoliative cheilitis, lichen planus, actinic cheilitis and lymphedema [76]. Some lesions seen in OFG, like aphtous ulcers and angular cheilitis, are seen also in otherwise healthy individuals.

2.2.6 TREATMENT

Treatment of OFG is challenging and must be tailored to each individual patient. Various options for treatment are reported (Table 2), but a gold standard OFG treatment is lacking.

Avoidance of identifiable allergens in food or the environment may help to manage the condition [28,39,46,47]. Improvement of OFG symptoms has been reported with cinnamon- and benzoate-free diet in 54-78% of patients with OFG, and 23% required no other treatments [63]. A low phenolic acid diet with micronutrient supplementation has given promising results in the treatment of OFG, but further studies of long-term outcomes are needed [77]. Topical treatments, including topical corticosteroids and topical calcineurin inhibitors are especially useful in

mild disease. Combining topical treatment to parenteral medication increases the effect [67]. Systemic and intralesional corticosteroids are the most popular therapy for OFG. Intralesional corticosteroids, triamcinolone for example, are felt to be safe and effective, and are favoured over systemic corticosteroids due to the well-known side effects of the latter [28]. Combined therapy with topical and intralesional corticosteroids or systemic medications was most effective to control the lesions of OFG [67]. Antihistamines provide no help for the symptoms of OFG [78]. Oral antibicrobials, including lymecycline, minocycline, roxithromycin, metronidazole, and atzithromycin may be effective in some patients [28]. Clofazimine and thalidomide have also been reported as effective therapy [28,66]. Tumor necrosis factor alpha (TNFα) inhibitors, infliximab and adalimumab, have shown promising results in the treatment of OFG, although the majority of patients lost responsiveness over the long term [28,79]. A recent study treated 60 OFG patients with azathioprine, which was significantly more effective in patients with concomitant CD [80]. The group did not recommend azathioprine as the primary treatment in patients with OFG alone [80]. Another study treated OFG patients with Exclusive Enteral Nutrition (EEN), which is a recognized treatment for induction of remission in CD in children [81,82]. A good clinical response to OFG symptoms was observed in 19 of the 29 children treated with EEN [82]. EEN may be an effective treatment option for children with OFG or OFG with CD [82]. Small case series and case reports have also found effective treatment outcomes in response to hydroxychloroquine, methotrexate, dapsone, and mycophenolate mofetil [28]. Surgery may be considered in disfiguring cases after the disease is stabilized with medical therapy.

Table 2. *Treatments for orofacial granulomatosis reported in the literature.*

Treatment	
Dietary	Systemic
Cinnamon- and benzoate free diet	Systemic corticosteroids
Avoidance diet (identifiable allergens)	Oral antimicrobials
Low phenolic acid diet	Clofazimine
Exclusive Enteral Nutrition (EEN)	Thalidomide
Topical	Tumor necrosis alpha (TNFα) inhibitors
Topical corticosteroids	Infliximab
Topical calcineurin inhibitors	Adalimumab
Intralesional	Azathioprine
Intralesional corticosteroids	Methotrexate
Triamcinolone	Dapsone
	Mycophenolate mofetil
	Surgery

2.2.7 MELKERSSON-ROSENTHAL SYNDROME

Melkersson-Rosenthal syndrome (MRS) is a rare disorder that is often referred to as one manifestation of OFG. The classic form of MRS consists of a triad of symptoms; recurrent facial palsy, recurrent orofacial or labial oedema, and plicated or fissured tongue (lingua plicata) [6]. All three symptoms are present only in a minority of patients. The symptoms can also manifest nonsimultaneously, and have intervals of months or years between them [6], which makes the diagnosis of this condition more challenging. The diagnosis can often only be determined after several years of follow-up. A monosymptomatic and more common form of MRS, with only orofacial or labial oedema, is called cheilitis granulomatosa (CG). Histology in MRS, including CG, shows non-caseating lymphoepithelioid granulomas identical to OFG [6,27,83].

The incidence of MRS is undefined [27]. MRS can affect patients of any ages, but typically it begins between the second and third decade of life [6,27,68,84,85]. Orofacial swelling is the most common manifestation of the disorder, and the most important diagnostic feature [6,27,83,84,86]. Oral involvement is common, and can include buccal and labial swelling, as well as gingival, palatal, and lingual manifestations [6,68]. Swelling of the supraorbital or infraorbital tissues and cheeks has been reported [6]. The swelling is usually nonerythematous, painless, and nonpruritic, and it typically resolves after the first appearance. After recurrences, the swelling may become more persistent and can lead to chronic, fissured and reformed swelling. Facial paralysis and fissured tongue are estimated to occur in approximately a third of patients [6], and the whole triad in a quarter of patients with MRS [87]. Facial palsy is caused by lower motor neuron paralysis. The palsy usually has a sudden onset, and is clinically indistinguishable from idiopathic Bell´s palsy. Compared to Bell´s palsy, facial palsy in MRS has a higher genetic predilection and recurrence tendency [88], as well as a worse prognosis [89].

As for OFG, the etiology of MRS is unknown. Various theories have been proposed, including infectious, autoimmune, allergic, and genetic. The relationship between MRS and CD has been proposed [90], but remains uncertain [6,17,84,91].

The diagnosis of MRS is clinical and based on patient´s history and clinical features. Neuroimaging examinations may be needed to exclude other diseases, for example neurosarcoidosis. Histologic evidence of granulomas confirms the diagnosis, but it is not mandatory for the diagnosis. As in OFG, the granulomatous changes are not always present in biopsies, and their absence does not exclude the diagnosis of MRS. If taken, the biopsy is advised to take during acute oedema for the best probability of diagnostic findings [27].

MRS is associated with additional clinical findings, which may help the diagnostics in oligo- or monosymptomatic cases. Hyperhidrosis, hypogeusia, acroparesthesia, hyperacusis, epiphora, migraine [6], trigeminal neuralgia, keratitis, psychotic episodes, uveitis [85], and tinnitus have been reported.

Treatment of MRS is challenging. There is no specific treatment and thus far the management is mostly empiric. Systemic corticosteroids are the most common current and noninvasive treatment [85].

2.3 OFG AND CROHN´S DISEASE

As stated earlier, the term OFG is used to describe patients with granulomatous lesions in the orofacial area without evidence of CD or other systemic disease. Patients with CD who have oral manifestations are often described as having oral CD (OCD). Patients with OFG who have developed CD are referred in the literature as OFG with CD. However, there is overlap between the definitions.

Oral manifestations in CD and OFG cannot be distinguished clinically or histopathologically. The relationship between these entities is not completely clear. Clinicians have debated whether OFG is an oral manifestation of CD, or a chronic inflammatory disease of its own. Factors predicting the development of gut disease in patients with OFG, and factors associated to oral manifestations in patients with CD, are sought in numerous studies.

It has been suggested that onset of OFG in childhood is predictive for the development of CD [38,63,92]. In addition, some clinical features are suggested to associate more to CD than OFG only. These include ulceration of the mucosa, involvement of the buccal sulcus, elevated inflammatory markers, fistulae formation, and pyostomatitis vegetans [15,17,63,92]. A study from the UK reported distinct clinical oral features in patients with OFG only and OFG with CD, the first having more lip involvement and the latter involvement of the buccal sulcus, ulcers, and mucosal scarring [63]. In a Swedish cohort of 29 patients, no significant difference in the oral phenotype was found between patients with OFG, with or without CD [62]. On the other hand, CD with concomitant OFG seems to represent a distinctive subtype of CD, that is characterized by extensive inflammation, perianal disease, and pronounced granuloma formation in the intestine [93].

Genotypic differences in OFG and CD have been reported. Nucleotide binding oligomerization domain containing protein 2 (NOD2) is a gene strongly associated with CD [94-96]. In a study of 29 patients, none of the 17 patients with OFG carried the NOD2 variant, whereas 4 of the 12 patients with oral CD carried a certain NOD2 variant [62]. A larger study of 201 patients with OFG found significant enrichment of NOD2 variants in patients with OFG and CD compared to the patients with OFG

only [80]. These findings support that genetic variants in NOD2 seem to be associated with OFG in patients with concurrent intestinal disease [62,80].

Anti-saccharomyces cerevisiae antibody A (ASCAbA) is noted as a serological marker for CD [97-99]. Assessing ASCAbA in serum and saliva in patients with OFG and CD, salivary IgA antibodies was higher in patients with oral disease, and serum IgA antibodies higher in patients with gut disease [50]. This suggests that serum ASCAbA may serve as a marker of coincident gut disease in patients with OFG, and as a screening marker for possible development of CD [50].

Calprotectin is a dimeric calcium-binding protein. It contributes approximately 60% of the cytosol in neutrophils [100,101]. Inflammatory processes in the gut result in accumulation of neutrophils in the intestinal mucosa, and this results in the release of calprotectin in the stools, where it can be measured [100,101]. Measurement of faecal calprotectin is considered as a reliable marker of intestinal inflammation with a good clinical sensitivity in differentiating organic and functional conditions [100]. It is not specific for IBD, but it has a high negative predictive value in ruling out IBD in undiagnosed, symptomatic patients [102]. In patients with diagnosed IBD, faecal calprotectin is used in monitoring, giving evidence of either relapse or mucosal healing [102,103]. Measuring faecal calprotectin can be useful in identifying underlying gut disease also in patients with OFG [104,105].

It has been suggested that OFG with gastrointestinal tract involvement may be an own entity separate from OFG and CD [15,25,28]. This theory was proposed in a study, where ileal or colonic abnormalities were found in 19 of 35 (54%) patients with OFG and no gastrointestinal symptoms. Only two of these patients had endoscopic findings typical for CD [15]. This theory suggests three entities: oral CD (gastrointestinal CD with oral involvement), OFG with gastrointestinal involvement (OFG with subclinical or asymptomatic gastrointestinal endoscopy changes), and OFG without gastrointestinal involvement [28].

3 AIMS OF THE STUDIES

OFG is a rare inflammatory condition affecting the orofacial area. Descriptive studies of OFG, its clinical presentation, management and long-term behaviour, are limited and typically consist of small number of patients. Precise diagnostics concidering the spectrum of OFG, including MRS and CG, is challenging and there is conciderable overlap in the classification. CD can cause oral manifestations similar to those in OFG, and the relationship between OFG and CD remains debated. Patients with orofacial symptoms can present to physicians in many specialities. Gaining more knowledge of this disease spectrum is needed for better recognition of the patients and to enable developing better treatments for these patients in the future.

The aim of this thesis was to investigate and compare conditions presenting with orofacial symptoms —OFG, CD, and MRS— and study the connection between these entities.

The specific aims of the studies are as follows :

I Evaluate and describe a cohort of pediatric OFG patients in detail, and study the long-term outcome of OFG, including dental and otorhinolaryngological status, and to evaluate possible factors predicting the development of CD in patients with OFG.

II Investigate the association of human leukocyte antigen (HLA) alleles and OFG.

III Examine the oral and otorhinolaryngological lesions and symptoms in patients with pediatric-onset CD when they reached adulthood, evaluate the factors associated with oral manifestations, study the long-term oral health outcomes and the health-related quality of life of patients with CD, and compare the findings to healthy controls.

IV Study the long-term health outcomes of patients with MRS having emphasis on the oral and intestinal symptoms, and investigate the incidence of CD in patients with MRS.

4 MATERIALS AND METHODS

4.1 STUDY I AND II

Patients diagnosed with pediatric OFG and treated between January 2000 and March 2014, at Helsinki University Children´s Hospital and department of Dentistry, Tampere University Hospital, Finland, were enrolled to this study performed between November 2013 and March 2014. The diagnosis of OFG was based on typical clinical features assessed by a dentist and a gastroenterologist and in most patients also a mucosal biopsy had been obtained showing granulomatous inflammation. The diagnosis of CD fulfilled the diagnostic Porto criteria including endoscopies with biopsies and small bowel imaging.

Altogether 29 patients (15 patients in Helsinki and 14 patients in Tampere) of the total of 40 traced patients agreed to the study.

Study visits (study I)

Patients were clinically examined using a structural schema by an otorhinolaryngologist (AH) and a dentist (HA/LA/US). The otorhinolaryngological examination (appendix 2) included fiberoscopic laryngoscopy. Findings in the face and mouth were recorded with digital photographing. Orofacial findings were carefully recorded using Oral disease activity score chart for OFG [36] (appendix 1). In the score, each site involved is scored individually for activity and type of lesion, with the total score indicating global severity, and the maximum total score being 87 [36]. The oral examination was carried out in a dental unit using a dental mirror, a dental and periodontal probe, fiber optic illumination, and appropriate dental lighting. Radiographs were not included in the study. The oral hygiene level was assessed according to the Silness-Löe plaque Index [106]. The periodontal condition was assessed by measuring the depth of periodontal pockets and the presence of bleeding after probing in all teeth. Dental caries were recorded with DMFT (decayed, missing, or filled teeth)-index [107].

Questionnaires (study I)

Patients, or in minors under the age of 18 years their guardians, were asked to complete questionnaires about general health, medication, preceeding use of antibiotics, bowel-related symptoms, orofacial symptoms, and special diets. To assess patients´ subjective opinion of their symptoms, patients were asked to evaluate the severity of their orofacial and bowel-related symptoms at the time of the study visit on a Likert scale with 7 response points, where 1 represents no symptoms and 7 very difficult symptoms.

To evaluate the patients´ nutrition, they were requested to keep a food record for 3 subsequent days before the scheduled study visit. The use of dietary supplements was also queried in the food records. A dietitian recorded the food records with a software that is based on a national database of foods. Information about given therapies was collected from the patient charts.

Laboratory investigations (study I)

Patients were requested to provide a stool sample for measuring faecal calprotectin to evaluate the possible inflammation in the gut. Calprotectin levels were measured in a routine clinical laboratory using a quantitative enzyme immunoassay (PhiCal Test, Calpro AS, Oslo, Norway), and values of <100 µg/g were considered to be normal [108,109]. Blood samples were collected from all patients to measure C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin, blood leukocyte count, and anti-Saccharomyces cerevisiae antibodies A (ASCAbA) and G (ASCAbG).

Laboratory investigations (study II)

To investigate the connection between HLA and OFG, the HLA-haplotypes from blood samples of the patients were analysed. Genomic DNA was extracted from EDTA-anticoagulated whole blood. HLA-haplotypes were constructed computationally with PHASE, a software that uses a Bayesian theorem [110]. Pediatric patients with CD (n=61) and the general population cohort (n=150) served as controls.

The Ethics Committee of Helsinki University Hospital approved the study and the subjects, or in minors under the age of 18 years their guardians, signed an informed consent form.

4.2 STUDY III

This case-control study was performed between September and December 2016. To study the oral manifestations in CD, Finnish-speaking adult patients from the Helsinki capital region who were born between 1987 and 1997 and diagnosed with CD in childhood after the year 2000 at the Helsinki University Children´s Hospital, Finland, were enrolled. As in study I and II, the diagnosis of CD fulfilled the diagnostic Porto criteria, including endoscopies with biopsies and small bowel imaging. The patients were invited to the study regardless of whether they had a history of oral CD or not.
Healthy controls matched for age and sex were collected from the Population Register Centre. The final numbers were 24 in the CD group and 22 in the control group.

Study visits

Patients and controls were examined as in study I using a structured schema by a dentist (HA/LA/US) and an otorhinolaryngologist (AH). The schema of the study visits and the equipment and instruments used were identical to those in study I.

Questionnaires

Questionnaires were used to evaluate subjects´ general health, medication, special diets, and lifestyle habits including smoking and alcohol comsumption. Health-related quality of life (HRQoL) was evaluated using the 15D questionnaire which is a generic, standardized, self-administered measure of HRQoL for individuals aged 16 years or older (appendix 3). The 15D includes the following 15 dimensions: breathing, mental function, speech (communication), vision, mobility, usual activities, vitality, hearing, eating, excretion, sleeping, distress, discomfort and symptoms, sexual activity, and depression. For each dimension there is one question, and each question has five possible responses. The 15D can be used as a profile instrument, but it can also provide a single index 15D score, ranging from a maximum of one when there are no problems on any dimension to the minimum of zero when the subject has died [111]. A change of 0.015 in the total 15D score is considered to be the minimum clinically important difference that a patient can feel [112].

To evaluate how patients experience their symptoms, the patients were requested to evaluate the severity of their gut symptoms and possible orofacial symptoms at the time of the examination, on a Likert-scale with 7 response points where 1 represents no symptoms and 7 very difficult symptoms.

Nutrition was evaluated from three-day food records as in study I. Information about given therapies was collected from the patient charts.

Laboratory investigations

Laboratory investigations were similar to those in study I.

The Ethics Committee of Helsinki University Hospital approved the study and the subjects signed an informed consent form.

4.3 STUDY IV

For the fourth study, interviews were pursued with Finnish-speaking adult patients with triad or oligosymtomatic forms of MRS or CG who had been treated and diagnosed with MRS in Helsinki University Hospital after the year 1995, and had participated in an MRS study in Helsinki University Hospital in 2007 [27]. The study cohort comprised 27 patients, 13 with triad or oligosymptomatic MRS and 14 with CG. This follow-up study was performed between May and July 2018.

Phone-interview

To evaluate the long-term outcome of patients with MRS, a phone interview was conducted with the patients. Patients were interviewed by an otorhinolaryngologist (AH) using a structural schema (appendix 4). The interview included a total of 42 questions regarding orofacial symptoms, facial palsy, intestinal symptoms, concomitant illnesses, medications, and possible food avoidances. In addition, family history of MRS, CG, and IBD was inquired. Additional information concerning the studied condition was collected from the patient charts.

Laboratory investigations

To evaluate the possible inflammation in the gut, the subjects were asked to provide a stool sample for measuring faecal calprotectin in a routine clinical laboratory using a quantitative enzyme immunoassay (PhiCal Test, Calpro AS, Oslo, Norway). Values of <100 μg/g were considered to be normal [109,113]. Stool samples were collected from 18 subjects.

The Ethics Committee of Helsinki University Hospital approved the study. All subjects signed an informed consent form.

4.4 STATISTICAL ANALYSIS

The nonparametric Mann-Whitney and Spearman correlation tests and the Fisher exact test were used to examine the associations between variables. In study II, Chi-square and Fisher exact test were applied, when appropriate.
Statistical analyses were performed using GraphPad Prism 6 (study I) and GraphPad Prism 7 (study III-IV) for Windows (GraphPad Software Inc., San Diego, CA) and SPSS software version 20 (study II) and SPSS software version 22

(analyses concerning nutrition in studies I and III. SPSS Inc., Chicago, IL). Differences were considered significant at p-value <0.05.

5 RESULTS

5.1 STUDY I

In this study, patients diagnosed with pediatric OFG were clinically examined by an otorhinolaryngologist and a dentist. Study included questionnaires and evaluation of nutrition.

Of the 29 patients, 21 (72%) had been diagnosed with CD during the time after the OFG diagnosis of median 3.1 years. Eleven patients had the OFG diagnosis before the CD diagnosis, and 5 patients got the diagnoses simultaneously (median time from OFG diagnosis to CD diagnosis 0.6 years, range 1 day – 4.6 years). Eight (28%) patients had not been diagnosed with CD. Patient characteristics are shown in Table 3.

Table 3. *Study I and II, Patient characteristics*

No. of patients	***29***
Male Female	22 (76%) 7 (24%)
Age, median, years (range)	
At OFG diagnosis	12.3 (2.9-15.8)
At Crohn´s disease diagnosis	12.3 (1.9-17.6)
At study appointment	14.3 (6.8-31.1)
Duration, years, median (range)	
Time after OFG diagnosis	3.1 (0.1-21.8)
Diagnoses	
OFG	29
Crohn's disease	21
Previous surgery	3
Fistulazing perianal disease	7
Fistulazing perianal disease with perianal abscess	4

Oral findings

All except one of the 29 patients had oral findings at the time of the study. The activity of patients´ orofacial lesions according to total score of oral disease activity score chart for OFG ranged between 0 and 33 (median 10) out of a possible score of 87. Orofacial findings were more severe in patients with CD and thus, total

scores higher compared to patients with OFG only (medians 11 and 7.5 respectively, p=0.0351). The oral mucosa was the most common site of orofacial findings in 26 (90%) patients, following lesions in the lower lip (76%, mostly swelling), gingival lesions (66%) and angular cheilitis (48%) (Figure 1.). Pictures of typical findings are shown in Figure 2. Between patients with CD and patients with OFG only, there was a statistically significant difference only in the number of patients who had upper lip swelling (p=0.0265) which was more common in patients with CD (Table 4). The prevalence of cobblestoning, fibrous banding/scarring, facial swelling, facial erythema, and staghorning, did not differ significantly between patients with OFG and CD and patients with OFG only (Figure 3).

Patients' subjective evaluation regarding orofacial symptoms at study visit varied from 1 (no symptoms, n=11) to 6 (difficult symptoms, n=1), median 2 and in CD, regarding bowel-related symptoms between 1 (no symptoms, n=6) to 5 (rather difficult symptoms, n=2), median 2.5.

Dental findings did not correlate statistically with the severity of the orofacial findings. All patients had gingivitis.

Otorhinolaryngological findings were minor in all patients (Table 5).

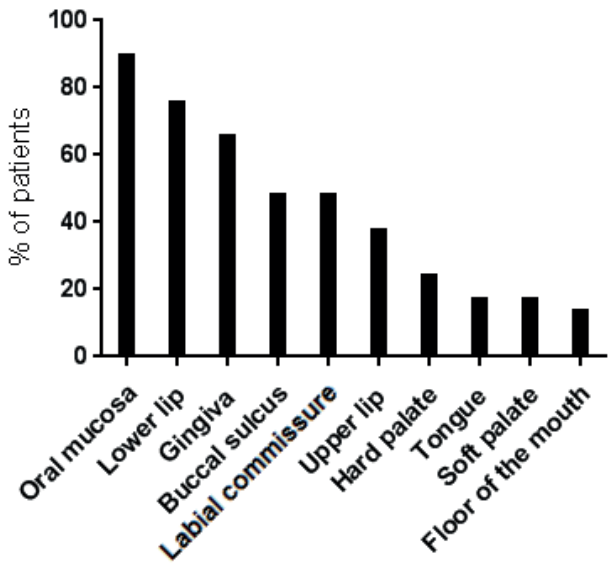


Figure 1. *Site of disease involvement in patients with OFG. Modified from the Journal of Pediatric Gastroenterology and Nutrition (Haaramo A et al: Detailed Follow-up Study of Pediatric Orofacial Granulomatosis Patients. J Pediatr Gastroenterol Nutr. 2017; 65(4): 388-393.)*

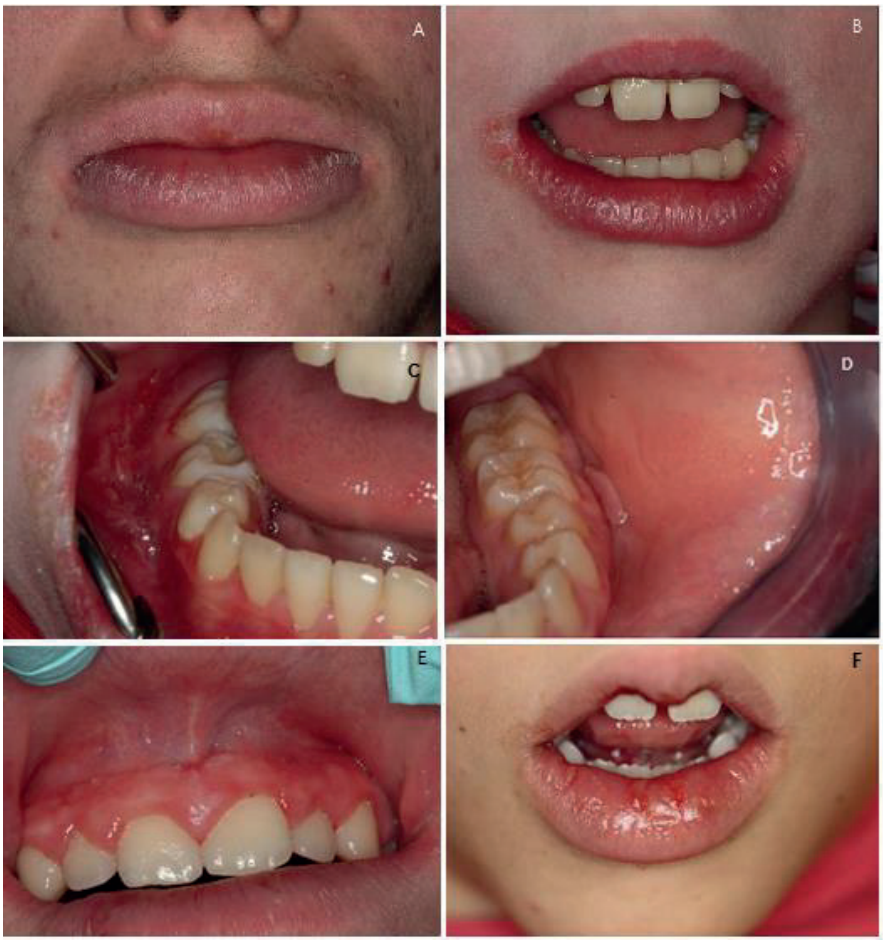


Figure 2. *Typical lesions in patients with OFG: Swelling of the lips (A, F), angular cheilitis (B), mucosal tags (C, D), and gingival enlargement (E).*

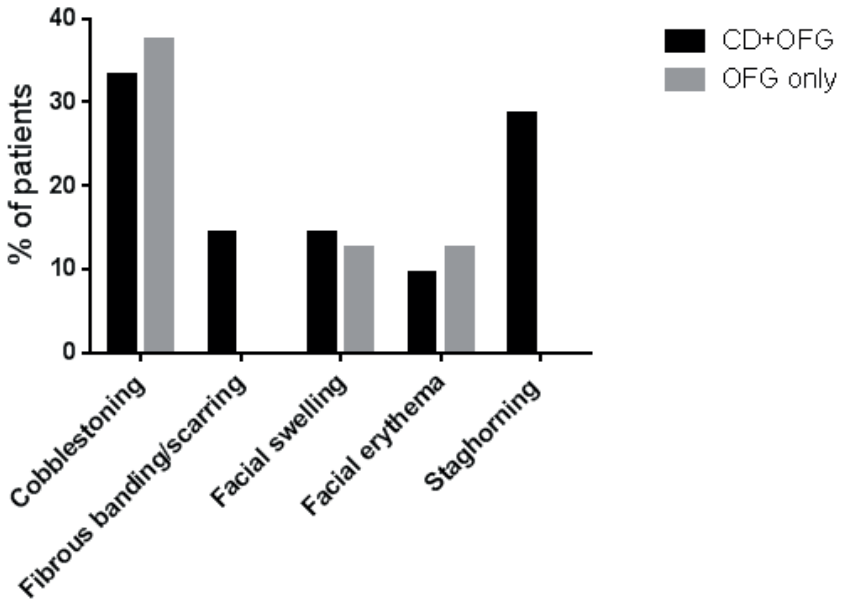


Figure 3. *Inflammatory findings in patients with OFG only (OFG only) and in patients with OFG and CD (CD+OFG).*

Table 4. *Site and type of disease involvement in patients with OFG and CD (OFG + CD) and patients with OFG only (OFG only). Modified from the Journal of Pediatric Gastroenterology and Nutrition (Haaramo A et al: Detailed Follow-up Study of Pediatric Orofacial Granulomatosis Patients. J Pediatr Gastroenterol Nutr. 2017; 65(4): 388-393.)*

No. of patients	***all 29 (%)***	***OFG + CD 21***	***OFG only 8***
Lips			
Lower lip	22 (75.9)		
swelling	19	15	4
erythema	7	5	2
ulcerations	1	1	0
Upper lip	11 (37.9)		
swelling	10	10	0
erythema	3	3	0
ulcerations	3	3	0
Angular cheilitis	14	12	2
Oral mucosa	26 (89.7)		
swelling	13	11	2
erythema	5	4	1
tags	6	4	2
ulceration	3	3	0
fissures	7	6	1
nodules	13	8	5
Buccal sulcus	14		
swelling	2	2	0
erythema	5	4	1
tags	5	4	1
ulceration	6	6	0
fissures	8	6	2
Gingiva	19 (65.5)		
swelling	10	8	2
erythema	16	13	3
ulcerations	0	0	0
nodules	4	4	0
Tongue	5 (17.2)		
swelling	0	0	0
erythema	2	2	0
ulcerations	2	2	0
fissures	1	1	0
Floor of the mouth	4 (13.8)		
swelling	0	0	0
erythema	1	1	0
tags	3	3	0
Soft palate	5 (17.2)		
swelling	2	0	2
erythema	1	0	1
nodules	3	3	0
Hard palate	7 (24.1)		
swelling	5	4	1
erythema	6	4	2
tags	1	1	0

Table 5. *Otorhinolaryngological findings in patients with OFG and CD (OFG+CD) and patients with OFG (OFG only). Modified from the Journal of Pediatric Gastroenterology and Nutrition (Haaramo A et al: Detailed Follow-up Study of Pediatric Orofacial Granulomatosis Patients. J Pediatr Gastroenterol Nutr. 2017; 65(4): 388-393.)*

No. of patients	***all 29***	***OFG+CD 21***	***OFG only 8***
Lymph nodes			
Enlargement of mental lymph nodes	1	0	1
Enlargement of submandibular lymph nodes	5	4	1
Enlargement of lateral cervical lymph nodes	1	1	0
Salivary glands			
Swelling of parotid glands	0		
Swelling of submandibular glands	0		
Nose			
Swelling of nasal conchae	0		
Atrophy of nasal conchae	0		
Erythema of nasal mucosa	6	4	2
Nasal polyps	0		
Septum deviation	1	0	1
Septum perforation	0		
Mucus in nasopharynx	2	1	1
Pharynx			
Undergone tonsillectomy	2	1	1
Tonsills			
gradus I	17	12	5
gradus II	10	8	2
gradus III-IV	0		
Chronic tonsillitis	0		
Larynx	26		
Swelling of laryngeal cartilages	3	2	1
Erythema of laryngeal cartilages	3	2	1
Swelling of epiglottis	0		
Erythema of epiglottis	1	1	0
Abnormal or dysfunctioning vocal cords	0		
Ears			
Swelling or secretion of ear canals	0		
Abnormal tympanic membrane	0		
Secretion in the middle ear	0		
Tympanogram	27		
A-curve	27	19	8
B-curve	0		
C-curve	0		

Treatment

The most common treatment for OFG in this study´s cohort was topical tacrolimus gel in 15 patients (51.7%). Any topical treatment was received by 17 (59%) patients and none was was received by 12 (41%) patients (10 patients with CD and 2 with OFG only). One patient with OFG had received methotrexate, while others receiving systemic medication were patients with CD. At study point 3 (10%) patients were using topical tacrolimus, 4 (14%) azathioprine, 3 (10%) anti-TNFα therapy (infliximab), and 2 (7%) methotrexate.

Laboratory investigations

Elevated levels of CRP over 3 mg/L [114] were measured in six patients (median 7.85, range 3.3-29.6 mg/L), all with CD. Elevated ESR was found in seven patients (median 25, range 13-38 mm/h), six with CD.

Twelve patients had elevated levels of ASCAbG (median 32, range 26-95 IU/L). Of these, 10 had CD. Six patients had elevated ASCAbA levels (median 45, range 20-167 IU/L) and were all diagnosed with CD. Interestingly, there was a significant relationship between elevated ASCAbA and patients´ total score of oral findings (p=0.0311) but no significant relationship between elevated ASCAbG and patients´ total score (p=0.0895).

There was no significant relationship between patients´ faecal calprotectin levels and oral disease activity (Spearman´s r=0.03744, n=29, p=0.8471). As expected, patients with CD had higher faecal calprotectin values than patients with OFG only (p=0.011).

Nutrition

Thirteen of the 28 patients that provided food records had eaten foods containing cinnamon. All except one patient had eaten food containing benzoic acid either in its natural forms or as an additive. Neither the consumption of cinnamon nor benzoic acid associated to orofacial findings (Spearman´s r=-0.2529, p=28, p=0.1942 and Spearman´s r=-0.06313, n=28, p=0.7496 respectively).

Most variation was seen in the intake of dietary fiber, but no significant correlation between patients' fiber intake and oral disease activity was found (p=0.7154).

Main results

Of the 29 patients with pediatric-onset OFG, 21 (72%) developed CD. Patients with OFG and CD had more oral findings than patients with OFG only. The oral mucosa was affected in 26 (90%) patients and was the most common site of orofacial findings. Swelling of the upper lip was more common among patients with CD than

patients with OFG only (p=0.0265), but other statistically significant differences in the orofacial findings between the groups were not found. Elevated levels of ASCAbA were found in patients with CD but not with OFG only. Patients with elevated levels of ASCAbA had also more oral findings compared to patients with normal levels of ASCAbA (p=0.0311).

5.2 STUDY II

In this study the HLA-haplotypes of patients with pediatric OFG were analysed to examine the relationship between HLA alleles and OFG. The results were compared between patients with OFG only and patients with OFG and CD. The groups were compared also to a cohort of pediatric patients with CD and to a general population cohort.

The patients with OFG only and OFG with CD did not differ significantly in the HLA-allele or HLA-haplotype frequency. When comparing the HLA-allele frequencies between patients with OFG and CD, and the control cohort of patients with pediatric CD, HLA-B*44 was more common in patients with OFG and CD than in all pediatric CD patients (7/42, 16.67% and 7/122, 5.74% respectively, p=0.049). Compared with the general population, there was increased frequency of HLA-B*44 in patients with OFG and CD, but statistical significance was not reached (7/42, 16.7% in patients with OFG and CD vs. 25/300, 8.7%, in general population, p=0.156). None of the other HLA-alleles or haplotypes associated with OFG.

Main results

One HLA-allele, namely HLA-B*44, was found to be associated with OFG, but not with CD.

5.3 STUDY III

This case-control study included clinical examination of adult patients with pediatric-onset CD. Evaluation of nutrition and health-related quality of life were included. Results were compared to matched healthy controls.

The study group comprised 24 adult patients with pediatric-onset CD and 22 healthy controls matched for age and sex. Patient characteristics are shown in Table 6. The median duration after the primary diagnosis of CD in the patients was 9.0 years (range 4.7-15.2 years).

Table 6. *Study III, patient characteristics*

Number of subjects	**46**
Patients with CD	24 (52.2%)
Male	14 (58.3%)
Previous surgery	5 (20.8%)
Fistulazing perianal disease	7 (29.2%)
Healthy controls	22 (47.8%)
Male	11 (50.0%)
Age in years, median (range)	
At Crohn´s disease diagnosis	13 (8-16)
At study appointment	
Patients with CD	22 (19-28)
Healthy controls	22 (19-29)
Duration of CD in years, median (range)	
Time between CD diagnosis and study	9.0 (4.7-15.0)

Oral findings

Of the 46 participants, 19 (41.3%) reported oral symptoms at some point of their life. Of the 24 patients with CD, 14 (58.3%) reported oral symptoms at some point during the course of the disease.

At the time of the study, the patients and controls had very few oral findings and the total scores for OFG, according to the oral disease activity score chart, ranged between zero and three (median 0) out of a maximum score of 87. Of the 46 subjects, 10 (21.7%) had findings in the orofacial area and these comprised eight (33.3%) of the 24 patients with CD and two (9.1%) of the 22 controls. All oral findings were recorded as mild. The most common oral finding was angular cheilitis in seven (15.2%) of the total of 46 subjects, including six with CD, but no statistical significance was reached between the controls and patients with CD (p=0.0984). None of the study subjects had cobblestoning, linear ulcers, fibrous banding/scarring, facial swelling, facial erythema, or staghorning. In addition, none of the patients had findings in their upper or lower lip, lower gingiva, tongue, floor of the mouth, hard palate, or pharynx. Four patients with CD who had oral findings at the time of the study had not reported any orofacial symptoms.

Patients with CD and a history of perianal abscesses had more oral manifestations than patients without perianal disease (Spearman´s r=0.45, n=23, p=0.0312). No correlation was found between the time from the primary diagnosis of CD and orofacial findings (Spearman´s r=-0.3059, n=24, p=0.1460).

The dental findings did not differ significantly between the patients with CD and controls. None of the dental findings correlated statistically significantly with the total score of oral manifestations. All except two subjects had gingivitis and only seven (15.2%) were cavity free with a DMTF score of zero.

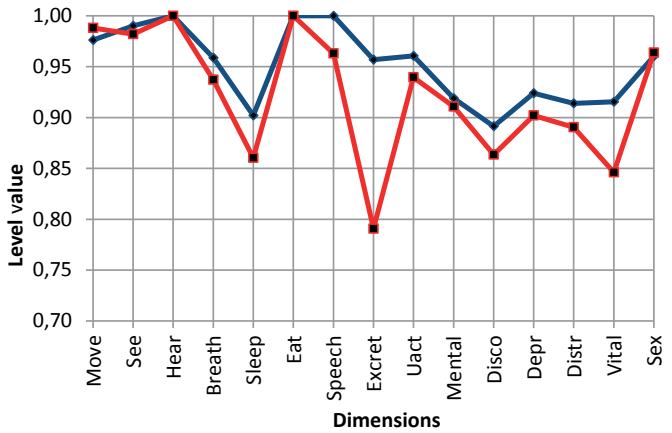
A comprehensive otorhinolaryngological examination was performed on all subjects but findings were very minor in both patients with CD and controls.

Treatment for CD

At the time of the study, 12 (50.0%) of the 24 patients with CD were treated with biological drug therapy and 17 (70.8%) had at some point of the disease course received biologicals. It did not have a significant effect on the oral findings wheter the patient received biologicals or not (Spearman´s r=0.01407, n=24, p=0.9480). Three patients had not received any medication for CD at the time of the study and there was no increase in their oral findings compared to the other patients with CD (p=0.9481).

Health-related quality of life

The mean HRQoL scores were lower in patients with CD, with a mean of 0.920 out of a possible score of one, compared with 0.951 for the controls. The difference was regarded as clinically important [112], but not statistically significant. There were slight differences between the groups in the dimensions of vision, breathing, sleeping, speech, excretion, usual activities, distress, depression, discomfort and symptoms, and vitality, as shown in Figure 4. However, the only statistically significant difference between the patients with CD and controls was found in the dimension of excretion ($p \leq 0.01$). When the subjects with oral findings, defined as a total OFG score of ≥1, were compared to those without oral findings, with a total OFG score of zero at the time of the study, subjects without oral findings fared better on every HRQoL dimension, except hearing, eating, and vitality. However, a statistically significant difference was found only in breathing ($p<0.05$), and subjects without oral findings had better scores. The disease duration in patients with CD did not affect the HRQoL significantly.



Move=mobility, See=vision, Hear=hearing, Breath=breathing, Sleep=sleeping, Eat=eating, Speech=speech (communication), Excret=excretion, Uact=usual activities, Mental=mental function, Disco=discomfort and symptoms, Depr=depression, Distr=distress, Vital=vitality, Sex=sexual activity

Figure 4. *Health-related quality of life in patients with pediatric-onset CD studied at adult age and controls, as evaluated by the 15D questionnaire. Modified from the Journal of Clinical Gastroenterology (Haaramo A et al: Oral and otorhinolaryngological findings in adults who were diagnosed with pediatric-onset Crohn´s disease: a controlled study. J Clin Gastroenterol. 2018 Jun 16 doi: 10.1097/MCG.0000000000001074. [Epub ahead of print]).*

Laboratory investigations

Twelve (26.1%) of the subjects had elevated CRP levels over 3 mg/L [114], but the patients with CD did not have higher CRP levels than the controls (Table 7). ESR was above the age related reference values [114] in seven (15.2%) of the 46 subjects, with a median of 5 mm/h (range 2-66 mm/h). ESR did not differ significantly between the patients with CD and controls and neither did the blood leukocyte (Table 7).

Neither the CRP values nor the blood leukocyte counts correlated with the presence of orofacial symptoms, which would have been indicated by higher OFG total scores (Spearmans r=-0.01183, n=43, p=0.9378 and Spearman´s r=0.2133, n=43, p=0.1546 respectively). Instead, higher ESR failed to correlate with higher total scores for orofacial findings (Spearman´s r=0.3363, n=43, p=0.0239).

Table 7. *Laboratory test results in patients with Crohn´s disease (CD) and in controls*

Laboratory test	***Results, median (range)***		
	CD	***control***	
CRP (mg/L)	1 (1-13)	1 (1-67)	p=0.1639
ESR (mm/h)	8 (2-39)	12 (2-66)	p=0.0631
Blood leukocyte count (E9/L)	6.9 (4.0-12.3)	6.9 (3.4-11.6)	p=0.5744
Faecal calprotectin (μg/g)	121.5 (6-2488)	19 (3-174)	p=0.002
CRP=C-reactive protein, ESR=erythrocyte sedimentation rate.			

Seven (15.2%) of the 46 subjects had elevated levels of ASCAbA (median titre 30 U/ml, range 23-156) and six of them had CD. Elevated levels of ASCAbG were found in six (13.0%) subjects (median 30 U/ml, range 27-63), including four who had CD. Elevated ASCAbA or ASCAbG levels failed to correlate significantly with the activity of subjects´ oral findings (Spearman´s r=-0.1012, n=46, p=0.5033 and Spearman´s r=0.07197, n=46, p=0.6346 respectively).

Fourteen (32.6%) of the 43 subjects who provided a stool sample had elevated (≥100 μg/g) levels of faecal calprotectin (median 308, range 100-2488 μg/g). As expected, patients with CD had significantly higher calprotectin levels than the controls (p=0.002, Table 7), but these were not associated with more oral findings in patients with CD (Spearman´s r=0.1819, n=24, p=0.3948). One healthy control without gut symptoms had faecal calprotectin above the normal range (174 μg/g), but this was back to normal within in a couple of weeks, and further tests were considered unnecessary.

Nutrition

Seventeen subjects (10 patients with CD (41.7%) and 7 controls (31.8%)) reported avoiding some food items in their diet. Avoiding lactose was most common (in seven subjects). Four patients (16.7%) and four controls (18.2%) reported food allergies.

The consumption of benzoic acid and cinnamon were recorded from the food records. All but three of the 46 subjects had eaten food containing benzoic acid, either in its natural forms, or as an additive. No association was found between the amount of benzoic acid consumption and activity of oral findings (p=0.4127, Spearman´s r=-0.1643). In addition, the proportion of subjects who consumed cinnamon did not differ between the patients with CD (37.5%) and the controls

(31.8%, p = 0.686) and there was no correlation between the amount of cinnamon consumption and activity of oral findings (p=0.1942, Spearman´s r=-0.2529).

No difference in energy and nutrient intakes was seen between the patients and controls: the median intakes of carbohydrates, fiber and folic acid were lower than recommended in the patients and controls and the median intakes of saturated fatty acids were higher than recommended [115]. The oral findings did not correlate with energy intake (r_s = 0.082, p=0.590), whereas those with moderate gastrointestinal symptoms received less energy than those with mild or no symptoms (22.9 vs 33.2 kcal/kg/day, p=0.042, t-test). However, there was no statistical difference in their body mass indexes, which were a median of 28.1 in the symptomatic subjects and a median of 23.6 in the subjects with mild or no symptoms (p= 0.150).

Smoking and alcohol consumption

Taking snuss (n=7) correlated with more orofacial findings and a higher total score (Spearman´s r=0.3343, n=46, p=0.0232), although the oral findings that were clearly caused by taking snuss, for example damage to the gingival mucosa in the exact place where the subject kept the snuss, were excluded from the total score count. However, smoking (n=3) did not correlate with more orofacial findings (Spearman´s r=-0.1472, n=46, p=0.3288). Alcohol consumption was modest in all of the subjects.

Main results

Of the 24 patients with CD, 14 (58.3%) reported oral symptoms during the course of the disease. At the time of the study, both the patients and controls had very few oral findings, and the total scores for OFG ranged between zero and three (median 0) out of a maximum score of 87. Statistically significant differencies in orofacial findings were not found between patients with CD and controls.
Patients with CD and a history of perianal abscesses had more oral manifestations than patients without perianal disease (p=0.0312).
The mean HRQoL scores were lower in patients with CD than in the controls. The only statistically significant difference between the patients with CD and controls was found in the dimension of excretion (p≤0.01).

5.4 STUDY IV

In this follow-up study, patients with MRS were interviewed to evaluate the long-term health outcomes of these patients. Faecal calprotectin was measured to investigate inflammation in the intestine.

The study group comprised 27 patients. Triad or oligosymptomatic MRS was diagnosed in 13 (48.1%) patients and the monosymptomatic form of the syndrome, CG, in 14 (51.9%) patients. At initial presentation of symptoms of MRS or CG the patients were aged 10-71 years (median 24 years), resulting in a median of 29.7 years follow-up. Patient characteristics are shown in Table 8.

Table 8. *Study IV, patient characteristics*

Number of subjects	**27**
Diagnoses	
Melkersson-Rosenthal syndrome	27 (100%)
Triad or oligosymptomatic form	13 (48.1%)
Female	7 (53.8%)
Male	6 (46.2%)
Monosymptomatic form (Cheilitis granulomatosa)	14 (51.9%)
Female	10 (71.4%)
Male	4 (28.6%)
Age in years, median (range)	
At study appointment	57.8 (41.4-87.0)
At diagnosis	38 (23-73)
At presentation of symptoms	24 (10-71)
Duration in years, median (range)	
Follow-up after diagnosis	15.4 (10.9-34.9)
Follow-up after presentation of symptoms	29.7 (11.4-63.7)

Symptoms of MRS

The prevalence of labial oedema, facial oedema, oedema elsewhere in the body and fissured tongue is listed in Table 9. During the course of the disease, 17 (63.0%) of the 27 patients had experienced swelling of the lips and 5 of them within the last year. Lip swelling was bilateral in 7 patients, while 5 patients reported swelling only in the lower lip and 5 only in the upper lip. In 10 patients, the labial oedema was

recurrent, while in 5 patients it had occurred only once, and in 2 patients it had become persistant. Nine patients (33.3%) had experienced facial oedema other than lips at some point of the follow-up, and 6 of them within the last year, 2 within the last month. In all except one patient, facial oedema had occurred recurrently. The location of the facial swelling varied between the patients, but seemed to always occur in the same place in individual patients. The area around the mouth was the most common site, reported by 3 (11.1%) patients. Six (35.3%) patients reported oedema also in other body parts and the site varied in every patient.

Of the 13 MRS patients with facial palsy, 9 (69.2%) had experienced recurrent episodes of facial palsy, while in four (30.8%) a palsy had occurred only once. In 6 patients the facial palsy had manifested on both sides of the face during different episodes, while in 7 patients the palsy had affected only either the left or right side of the face. None of the patients with an original diagnosis of CG reported facial palsy.

Of the 13 patients with MRS and facial palsy history, 9 (69.2%) had a fissured tongue. The complete symptom triad of MRS was found in 4 (14.8%) patients.

Oral symptoms

Of the 27 study participants, 15 (55.6%) reported some oral symptoms during the course of the disease. Aphtous ulceration or blistering was the most common complaint in the mouth in 9 (33.3%) patients. Actual ulcers in the mouth were reported in 3 patients. Erythema of the oral mucosa was reported by 3 patients and staghorning (extended nodules) by 2 patients. Angular cheilitis was experienced at some point of the disease course by 7 (25.9%) patients.

Treatment for MRS and CG

As a treatment for facial palsy, eight (61.5%) of the 13 MRS patients with facial palsy had received peroral cortisone at least once. Of the 14 patients with CG, six (42.9%) had received peroral cortisone for oedema. Cortisone injections, laser treatment, topical tacrolimuse (calcineurin inhibitor) and surgery were each received by one (7.1%) patient with CG as a treatment for oedema.

Table 9. *Symptoms in patients with triad or oligosymptomatic Melkersson-Rosenthal syndrome and cheilitis granulomatosa during the course of the disease (ever) and within the last year before the study interview (last year) as self-reported by the patients.*

Number of patients	***27***	
	ever	***last year***
Facial oedema	9 (33.3%)	6(22.2%)
recurrent	8 (88.9%*)	6(66.7%*)
nonrecurring	1 (11.1%*)	0
bilateral	4 (44.4%*)	3(33.3%*)
unilateral	5 (55.6%*)	3(33.3%*)
location of facial oedema		
nose and close to nose	2 (22.2%*)	2(22.2%*)
jaw	2 (22.2%*)	0
around the mouth	3 (33.3%*)	2(22.2%*)
neck, cheek, under the eyes	1 (11.1%*)	1(11.1%*)
Labial oedema	17 (63.0%)	5 (18.5%)
recurrent	10(58.9%**)	2(11.8%**)
nonrecurring	5(29.4%**)	1(5.9%**)
persistent	2 (11.8%**)	2(11.8%**)
both lips	7 (41.2%**)	2(11.8%**)
upper lip	5 (29.4%**)	1(5.9%**)
lower lip	5 (29.4%**)	2(11.8%**)
bilateral	12(70.6%**)	4(23.5%**)
unilateral	5 (29.4%**)	1(5.9%**)
Oedema elsewhere	6 (22.2%)	0
Facial palsy	13 (48.1%)	1 (3.7%)
recurrent	9(69.2%***)	1(7.7%***)
nonrecurrent	4(30.8%***)	0
Fissured tongue	11 (40.7%)	

*percentage of all facial oedema
**percentage of all labial oedema
***percentage of all facial palsy

Bowel symptoms

Of the 27 patients, three (11.1%) were diagnosed with IBD; two MRS patients with UC, and one CG patient with CD. In addition, three patients were diagnosed with a functional condition, namely irritabile bowel syndrome. Six patients also had required clinical examination due to intestinal symptoms, but with negative results. None of the clinical features queried in the interview correlated significantly to the occurrence of bowel disease. The patient with CD presented first

with IBD, and was diagnosed with CD at the age of 27, after which she developed CG at the age of 35. Both patients with UC had MRS with facial palsy, which had presented before the symptoms of the gut disease.

Laboratory tests

Stool samples were collected from 18 patients to investigate the presence of intestinal inflammation using faecal calprotectin as a surrogate marker. Faecal calprotectin was above the normal level (>100μg/g) in 3 (16.7%) patients. Two of the patients with elevated levels of calprotectin had IBD. One non-IBD patient without intestinal symptoms had marginally elevated faecal calprotectin (144 μg/g) and was referred to control the value, but we are not aware of the result.

Diet

When queried about nutrition, 20 (74.1%) patients reported avoiding some food items in their diet. Eight (29.6%) patients reported avoiding certain food substances because they believed these substances were connected to oral or facial symptoms. Tomato, mustard, seasoning, ketchup, chocolate, kiwi, bell pepper, chili, food additives, and fruits and berries were mentioned to cause or exacerbate oedema in the lips (n=4), ulceration in the angle of the mouth (n=1) or aphtous ulceration in the mouth (n=2). One patient reported avoiding foods that contain nitrites due to causing facial oedema.

Main results

Of the 27 patients, 15 (55.6%) reported oral symptoms at some point of the disease course. Eight (29.6%) patients reported avoiding food items due to causing oral or facial symptoms. Two MRS patients were diagnosed with UC and one CG patient was diagnosed with CD. Altogether 11.1% of the patients were diagnosed with IBD. Only one non-IBD patient had marginally elevated faecal calprotectin, other two patients with elevated levels of faecal calprotectin had IBD.

6 DISCUSSION

6.1 OFG AND CD

One of the main aims of this thesis was to investigate the connection between OFG and CD, and to evaluate the possible factors predicting the underlying or developing gut disease in patients with OFG.

The largest study to date on OFG included 207 patients, who were traced retrospectively, including 22% of children [63]. The majority of the patients did not have CD nor develop it during the median of 5.5 years follow-up [63]. However, children who presented first with oral symptoms were more likely to develop CD than if the oral symptoms started in adulthood [63]. A review of 104 patients with pediatric OFG reported CD in about 40% of the patients, CD usually occurring before the onset of OFG [92]. In this study on patients with pediatric OFG, the frequency of CD was even higher, as the majority (72%) of the patients developed CD. In this study´s cohort, most patients presented first with OFG, and CD manifested later. Thus, it seems that pediatric-onset OFG may differ in clinical presentation from adult-onset disease. Childhood onset seems to carry a higher risk of developing CD in follow-up. High prevalence of CD in this study´s cohort may also be partly explained by patient selection. Patients were collected from two University Hospital units, where the most complicated cases are treated.

Faecal calprotectin is acknowledged to be a noninvasive marker for intestinal inflammation [104,105]. It is used to estimate the activity of the gut disease in patients with IBD [102], and to identfy inflammation in the gut in patients with bowel symptoms. This study´s findings support the understanding that faecal calprotectin is useful also in identifying underlying gut disease in patients with OFG. Patients with CD had, as expected, significantly higher levels of faecal calprotectin than patients with OFG only.

Concerning patients with MRS, this was the first study to systematically investigate the possibility of inflammation in the gut by measuring faecal calprotectin in these patients. Only one non-IBD patient with a marginally elevated value of faecal calprotectin was found. Several patients had experienced intestinal symptoms, but these patients did not have elevated levels of faecal calprotectin above 100µg/g, which would have indicated intestinal inflammation. Therefore, extensive screening for IBD in patients with MRS in the absence of gastrointestinal symptoms seems not beneficial, which is in line with earlier recommendations [91]. Nevertheless, it must be kept in mind that gastrointestinal inflammation is not completely ruled out when faecal calprotectin is between 50 and 99µg/g. In this study´s cohort, three patients, who did not have IBD, had faecal calprotectin

above 50 but under 100µg/g. Their calprotectin values were 55, 57 and 87µg/g, but only one of them reported intestinal symptoms. This patient group should be concidered for careful follow-up and controlling of faecal calprotectin if intestinal symptoms develop or proceed.

Three (11.1%) of the 27 patients with MRS or CG were diagnosed with IBD, one patient (3.7%) with CD and two patients (7.4%) with UC. The reported prevalence of IBD in Finland was 595 per 100 000 inhabitants in 2008 [13], and the current frequency of IBD in the Finnish population is 0.87% [116]. Thus, the prevalence of IBD was higher in this study´s cohort of patients with MRS or CG (11.1%) than in the general population, suggesting a link between MRS and IBD. A connection between MRS and CD has been described before, but a connection between MRS and UC is a novel finding, which has not been reported in the literature previously.

In addition to faecal calprotectin, it has been suggested that it may be possible to use ASCAbA, a known serological marker for CD [97,98], as a marker of coincident gut disease in patients with OFG, and as a screening marker of the possible development of CD [50]. In this study on patients with pediatric OFG, patients with elevated ASCAbA were all diagnosed with CD. In addition, patients with elevated ASCAbA, but not those with elevated ASCAbG, had more orofacial findings than patients with normal ASCAbA levels. Thus, elevated ASCAbA levels might predict more severe oral disease and ASCAbA might arise as a serological marker to detect underlying or developing CD in patients with OFG. Further studies are needed to ensure the connection.

Human leukocyte antigen (HLA) genes are associated with CD, although their overall effect on the pathogenesis of CD is not well established. HLA-B*44 is a class I HLA molecule that presents antigenic peptides to cytotoxic CD8+ T-cells [32]. The HLA-B*44 allele has previously been associated with type 2 polyarticular peripheral arthropathy in patients with IBD [117]. In this study, a possible connection between HLA-B*44 and OFG was found. Thus, HLA-B*44 may be a marker for OFG, and possibly a marker for extra-intestinal manifestations, including arthropathy and oral manifestations, in IBD. On the other hand, the finding that OFG with CD differed significantly from overall CD by having an increased frequency of HLA-B*44, suggests that OFG is not merely a manifestation of CD, but more likely a separate disease entity. Similar suggestion was made based on a small study from the UK, in which an association between OFG and HLA genotypes A3, B7 and DR2 was found [35]. The difference in their finding and this study´s finding rises the question of a possible geographical difference in the genetics of OFG.

Exact diagnosing in the field of OFG is challenging. Different conditions and their subtypes, like OFG, MRS and CG as well as oral manifestations in CD, have similar clinical features and findings, and distinctive features can in many cases manifest

not until after months or years of follow-up. This makes exact diagnosing difficult or even impossible, and inevitably leads to overlap in subgroups in studies. Therefore it may not be easy to find differences between subgroups. In addition, different studies are therefore not easily comparable. This may be one factor to explain the broad differences in incidences of many findings concerning these conditions reported in the literature. In this study, patients were collected from the same one or two tertiary care departments to minimize variation in classification and diagnostics, and thus to gain as comparable subgroups as possible.

6.2 ORAL FINDINGS

6.2.1 ORAL MANIFESTATIONS IN CD

A prospective study of pediatric patients with suspected CD found oral pathology in approximately 40% of the patients, and these mostly went unnoticed before they were examined [118]. This led to the evaluation of the presence of oral manifestations in patients with pediatric CD when they reached adulthood. No detailed description of oral manifestations in pediatric-onset CD in adulthood has been published previously.

More than half (58.3%) of the 24 patients with pediatric-onset CD in this study reported oral symptoms at some point in the course of the gut disease. At the time of the study, one third of the patients with CD had oral lesions. However, these were mostly isolated, and recorded as mild. The patients were receiving good therapeutic control for their gut disease during the study period, which may explain the reasonably low number of oral lesions. Thus, it seems that pediatric CD confers only a modest risk of oral lesions at adult age, although oral lesions were more common in the patients with CD than the controls, affecting one-third and one-tenth, respectively.

A study by Laranjeira et al. with 113 adult patients with IBD – either UC or CD – and 58 controls, found that oral lesions increased over the course of the gut disease [119]. In this cohort, the length of follow up ranged from 4.7 to 15.2 years after the CD diagnosis, and the time period was not associated with oral findings. In the Laranjeira et al. study, patients in clinically active phases of IBD were the most affected [119], but in a study with child patients this kind of correlation was not found [17]. This study of patients with pediatric-onset CD who had reached adulthood showed no correlation between the oral findings and the subjective evaluation of the severity of current gut symptoms. In addition, there was no correlation between oral findings and levels of faecal calprotectin. Similarly, in 29 patients with pediatric-onset OFG, including 72% who had CD, there was no

association between faecal calprotectin and orofacial findings. This suggests that oral inflammation and intestinal inflammation are driven by different, but still uncharacterized, factors. In the future it would be interesting to examine the mouths of patients with CD during the active phases of the gut disease.

A specific risk factor for oral findings in CD in adulthood was a history of perianal abscesses, which is an indicator of a complicated disease subtype of CD [120]. Others have also reported such an association between perianal CD and oral findings [17,23,118,121,122]. In children it has been suggested that the presence of oral manifestations, especially at the presentation of the gut disease, but presumably not later during the course of the disease, might be a marker for a severe CD subtype [17]. Therefore, it may be beneficial for the clinician to look in the mouths of patients with CD to find clues of a possible severe disease subtype. The oral cavity is easily accessible and can help clinicians to detect those patients who are in need of more accurate follow-up of the gut disease. Further studies with preferably larger cohorts are needed to ensure the true possibilities of the examination of the oral cavity in terms of assessing the circumstances in the gut.

6.2.2 CLINICAL PRESENTATION OF ORAL FINDINGS IN CD AND OFG

The spectrum of oral lesions reported in patients with CD varies widely. Cobblestoning, followed by linear ulcers have been reported to be the most common oral lesions in patients with CD [20]. The lips [21] and the labial commissure [22] have been reported to be the most frequently affected sites. In reviews on pediatric patients with CD, mucogingivitis [17] and oral ulceration [18] were the most common oral lesions. In this cohort of adult patients with pediatric-onset CD, angular cheilitis was the most frequently seen oral manifestation, while all other lesions were sporadic. Angular cheilitis was also common in patients with OFG, and was found in almost half of the patients. In patients with MRS, angular cheilitis was reported in a quarter of the patients. However, angular cheilitis is not specific to OFG or CD and it is seen quite commonly in otherwise healthy individuals. In patients with OFG, the most common findings according to the literature are lip swelling and facial swelling, reported in more than 90% of cases [64,66]. In this study the most common individual finding was swelling of the lower lip, which was present in about 65% of the patients with OFG. However, the most common site for orofacial lesions was the buccal mucosa, where almost 90% of the patients had some findings. The only statistically significant difference between patients with OFG and CD and patients with OFG only was the more frequent swelling of the upper lip in patients with concurrent CD. Concerning all other orofacial findings, no statistically significant differences between these groups were found.

Patients with OFG had plenty of oral findings at the time of the study, but stated that they were mostly asymptomatic. Altogether, evaluation of patients with pediatric-onset OFG after 3.1 years (median) after diagnosis, showed good prognosis and severe disease exacerbations were not detected. Interestingly, patients with OFG who had CD had more oral findings compared to patients with OFG only.

Over half of the patients with MRS reported lesions in the mouth at some point of their disease, the proportion being similar to patients with pediatric-onset CD in adulthood. Aphtous ulceration and blistering were the most common lesions in the mouth in patients with MRS. Some of the patients reported that symptoms in the mouth coincide with the facial oedema, while others had not noticed a time connection between the oedema and ulceration in the mouth. Interestingly, over half of the patients who reported oral lesions, had noticed that certain food substances cause or exacerbate the symptoms and therefore avoided those substances in their diet. Allergens in food seem to have a role in oral and labial symptoms of MRS as self-reported by the patients, but further studies on this topic are needed.

Biopsy and histopathological examination are not mandatory in the diagnosis of OFG, which is based on the typical clinical presentation of oral manifestations. Similarly, in MRS the diagnosis is clinical and biopsy is not necessary although the granulomatous changes would confirm the diagnosis. However, a biopsy is needed in certain cases if the possibility of a malignancy as a diagnostic option emerges. The possibility of a malignancy must be kept in mind if an oral lesion is not typical or does not respond well to treatment. In such cases, a biopsy is naturally mandatory and of great value.

6.3 OTORHINOLARYNGOLOGICAL FINDINGS

Otorhinolaryngological findings in patients with OFG or CD have not been reported in detail before. Surprisingly the otorhinolaryngological findings of patients were minor in these cohorts, both in patients with pediatric-onset OFG and in adult patients with pediatric-onset CD. Most subjects had normal findings in comprehensive otorhinolaryngological examination, which included fiberoscopy of the nasopharynx and larynx. This strengthens the assumption that OFG is restricted in the oral cavity and area around the mouth and does not affect the nasal mucosa, nor the larynx or the ears. In patients with CD signs of acute or chronic inflammation were not detected, which goes together with the assumption that inflammatory changes in CD are restricted to the gastrointestinal tract without excess otorhinolaryngological comorbidity. Based on clinical experience, patients with CD seem to suffer quite commonly from nasal obstruction, but neither the

patients´ own evaluation of nasal obstruction, nor the clinical findings of the nasal cavity and nasal conchae, differed significantly between the patients with CD and controls. In addition, nasal obstruction causing nasal polyps were not detected. A detectable adenoid was as common in the CD group as in the controls, but interestingly all patients with a mucous adenoid had CD.

The involvement of salivary glands in OFG has been suggested based on the finding that non-specific immunoglobulin levels in both whole saliva and parotid saliva were raised in patients with OFG with or without gut inflammation [50]. In this study, the clinical examination (palpation) of salivary glands was normal suggesting that salivary glands were unaffected. Subclinical involvement is naturally not precluded. However, if OFG patient´s salivary glands are affected, sarcoidosis should be considered in differential diagnosing as an uncommon cause of orofacial lesions, similar to OFG [123]. Inflammation in the oral cavity would be expected to result in enlargement of the cervical lymph nodes, but this was seen only in few patients in this study, although oral findings were seen in most of the patients with OFG.

Patients with OFG can primarily present to an otorhinolaryngologist with their various orofacial symptoms and otorhinolaryngologists should be familiar with this entity and remember the possibility of an underlying gut disease.

6.4 STRENGTHS AND LIMITATIONS

A valid strength of this series of studies is comprehensiveness. The studies include otorhinolaryngological examination, dental examination, nutritional aspects, laboratory tests, and evaluation of health-related quality of life. Orofacial manifestations are evaluated from many different aspects.

Otorhinolaryngological findings of patients with OFG and patients with pediatric-onset CD in adulthood have not been published before. It was surprising that there were so few otorhinolaryngological findings.

Nutrition was evaluated from three-days food records. Keeping a food diary requires writing down precisely everything that is eaten. This can be challenging for anybody and it contains a risk for errors. Underreporting is an acknowledged finding in the food records [124]. In children, the guardian made the markings to the food diary, but it is likely that the guardian was not present in every eating situation if the patient was in school or in daycare, which increases the risk for errors. To minimize the errors, a trained nurse checked the food records upon returning for possible omissions and controversies. Use of an electronic food diary that the patient can complete with a smartphone or another mobile device and that

alerts for obscurity, may decrease the possibility for omissions in food records in the future.
In study IV, a phone interview was performed. The symptoms were therefore self-reported by the patients which includes the possibility of recall bias. Patients´ experience in recognizing and evaluating the symptoms may vary between individuals, and it can affect reporting. However, performing the study as a phone interview presumably increased the number of participants. In addition, the advantages of the phone-interview were that defining questions could be asked of the patients if needed, and long-distance patients and patients from older age groups, who may find filling questionnaires or attending study visits difficult, could be reached. A proper clinical examination of the symptoms is difficult to arrange due to long symptom-free intervals in MRS.

In study IV, the long follow-up time after both presentation of symptoms and diagnosis is a valid strength. This is the longest follow-up of patients with MRS published to date. In addition, evaluating the possibility of underlying inflammation in the gut using faecal calprotectin as a surrogate marker has not been systematically done in patients with MRS before.

The relatively small number of patients is a limitation in the studies. In study III, recruiting young adults to the study turned out to be more challenging than expected. In study IV however, considering the rare nature of MRS and the long follow-up time, the number of patients included in the study was fairly good.

6.5 FUTURE PROSPECTIVES

The role of gut microbiota in immunity-related disorders is increasingly recognized. The strong modification of gut microbiota composition is the most important environmental factor associated with the development of CD [8]. Examining the composition of gut microbiota in patients with OFG is of particular interest for future research, and would likely provide new insights to etiology and treatment. Comparing the microbiota of patients with orofacial disease and those with CD, as well as patients with MRS, would advance knowledge of the relationships between these conditions. In addition to gut microbiome, the role of oral microbiome is of high interest. The oral microbiome is a direct precursor of oral conditions such as dental caries and periodontitis [125]. The influence of oral microbiome can extend beyond the oral cavity and systemic conditions such as coronary artery disease and rheumatoid arthritis are associated with oral microbiome [126,127]. The role of oral microbiome in the development of OFG and CD is also an interesting issue for future research [128].

To draw more precise conclusions of the clinical use of ASCAbA as a factor predicting the underlying or developing CD in patients with OFG, the results of this

thesis should be tested in preferably larger patient cohorts. In addition, the true meaning and clinical use of tissue antigen HLA-B*44 in OFG needs to be further evaluated.

7 CONCLUSIONS

OFG remains a rare entity. The study shows that prognosis for OFG is good. Symptoms tend to diminish during the course of the disease and topical treatment seems to be efficient and safe. When accompanied by CD, the oral symptoms seem to be inactive when the gut disease is under good therapeutic control. Pediatric CD confers only a modest risk of oral lesions at adult age.

In addition to faecal calrotectin, elevated serum ASCAbA may serve as a factor predicting the underlying or developing CD in patients with OFG, but further studies are warranted.

Tissue antigen HLA-B*44 might be a marker for OFG. On the other hand, patients with CD and a history of perianal abscessing disease seem to be prone to develop oral manifestations of CD. In addition, elevated ASCAbA also connects to more severe oral manifestations in patients with OFG with or without CD, but further studies are needed to ensure the connection.

OFG or CD does not seem to increase otorhinolaryngological comorbidity.

Oral manifestations in MRS do not seem to increase during the course of the disease, although over half of the patients reported oral symptoms at some point of the disease. Incidence of IBD was higher among the patients with MRS than reported in the Finnish general population. This suggests a link between MRS and IBD, not only CD but also UC.

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APPENDICES

APPENDIX 1. THE ORAL DISEASE ACTIVITY SCORE CHART FOR OFG

Site	Site score	Activity of lesion(0-3) Double if site= 2	Type of lesion	Type of lesion legend	
Upper lip (1)				1	Oedema
Lower lip (1)				2	Tags
Angular cheilitis (1)				3	Ulceration
Buccal mucosa left (<50% = 1, >50% = 2)				4	Linear ulcers
Buccal mucosa right oikea (1 or 2)				5	Erythema
Buccal sulcus left (1 or 2)				6	Fissures
Buccal sulcus right (1 or 2)				7	Nodules
Gingiva (1 each segment)					
Lower right (distal)					
Lower central					
Lower left (distal)				**Activity legend**	
Upper right (distal)				0	Absent
Upper central				1	Mild
Upper left (distal)				2	Moderate
Dorsum of the tongue (1 or 2)				3	Severe
Lateral tongue right (1)					
Lateral tongue left (1)					
Floor of the mouth (1 or 2)					
Hard palate (1 or 2)					
Soft palate (1 or 2)					
Pharynx (1 or 2)					
TOTAL					

Other findings:	**Yes/No:**
Cobblestoning	
Fibrous banding/scarring	
Facial swelling	
Facial erythema	
Staghorning	

APPENDIX 2. SCHEMA FOR OTORHINOLARYNGOLOGICAL EXAMINATION

Inspection of the face

	oedema	erythema
around the mouth	☐ yes ☐ no	☐ yes ☐ no
cheeks	☐ yes ☐ no	☐ yes ☐ no

comments:

Palpation of the neck

	right	left
enlarged lymph nodes		
mental region	☐ yes ☐ no	☐ yes ☐ no
submandibular region	☐ yes ☐ no	☐ yes ☐ no
lateral cervical region	☐ yes ☐ no	☐ yes ☐ no

Salivary glands

	right	left
Parotid glands		
oedema	☐ yes ☐ no	☐ yes ☐ no
pain	☐ yes ☐ no	☐ yes ☐ no
Submandibular glands		
oedema	☐ yes ☐ no	☐ yes ☐ no
pain	☐ yes ☐ no	☐ yes ☐ no

Anterior rhinoscopy

	right	left
conchae		
oedema	☐ yes ☐ no	☐ yes ☐ no
atrophy	☐ yes ☐ no	☐ yes ☐ no
mucosa		
pale	☐ yes ☐ no	☐ yes ☐ no
redness	☐ yes ☐ no	☐ yes ☐ no
polyps	☐ yes ☐ no	☐ yes ☐ no
septum		
deviation	☐ yes ☐ no	
septum perforation	☐ yes ☐ no	

Pharynx

tonsils ☐ gradus I ☐ gradus II ☐ gradus III
tonsilloliths ☐ yes ☐ no
coating ☐ yes ☐ no
comments:

Posterior rhinoscopy

adenoid ☐ yes ☐ no
comments:

Nasofiberoscopy

	right	left
vocal cords		
erythema	☐ yes ☐ no	☐ yes ☐ no
oedema	☐ yes ☐ no	☐ yes ☐ no
movement	☐ yes ☐ no	☐ yes ☐ no
normal	☐ yes ☐ no	☐ yes ☐ no
arythenoids		
oedema	☐ yes ☐ no	☐ yes ☐ no
erythema	☐ yes ☐ no	☐ yes ☐ no
normal	☐ yes ☐ no	☐ yes ☐ no

epiglottis
oedema ☐ yes ☐ no
erythema ☐ yes ☐ no

Ears

	right	left
Ear canal		
secretion	☐ yes ☐ no	☐ yes ☐ no
oedema	☐ yes ☐ no	☐ yes ☐ no
erythema	☐ yes ☐ no	☐ yes ☐ no
Tympanic membrane		
normal	☐ yes ☐ no	☐ yes ☐ no
perforation	☐ yes ☐ no	☐ yes ☐ no
Middle ear		
secretion	☐ yes ☐ no	☐ yes ☐ no

comments:

Tympanometer

	right	left
A-curve	☐	☐
B-curve	☐	☐
C-curve	☐	☐

not done ☐

Tuning fork tests

Rinne (+/-) right ☐ left ☐

Weber ☐ middle
☐ right
☐ left

APPENDIX 3. THE 15-DIMENSIONAL MEASURE OF HEALTH-RELATED QUALITY OF LIFE (15D)

Please read through all the alternative responses to each question before placing a cross (x) against the alternative which best describes your present health status. Continue through all 15 questions in this manner, giving only one answer to each.

QUESTION 1. MOBILITY
1 ( ) I am able to walk normally (without difficulty) indoors, outdoors and on stairs.
2 ( ) I am able to walk without difficulty indoors, but outdoors and/or on stairs I have slight difficulties.
3 ( ) I am able to walk without help indoors (with or without an appliance), but outdoors and/or on stairs only with considerable difficulty or with help from others.
4 ( ) I am able to walk indoors only with help from others.
5 ( ) I am completely bed-ridden and unable to move about.

QUESTION 2. VISION
1 ( ) I see normally, i.e. I can read newspapers and TV text without difficulty (with or without glasses).
2 ( ) I can read papers and/or TV text with slight difficulty (with or without glasses).
3 ( ) I can read papers and/or TV text with considerable difficulty (with or without glasses).
4 ( ) I cannot read papers or TV text either with glasses or without, but I can see enough to walk about without guidance.
5 ( ) I cannot see enough to walk about without a guide, i.e. I am almost or completely blind.

QUESTION 3. HEARING
1 ( ) I can hear normally, i.e. normal speech (with or without a hearing aid).
2 ( ) I hear normal speech with a little difficulty.
3 ( ) I hear normal speech with considerable difficulty; in conversation I need voices to be louder than normal.
4 ( ) I hear even loud voices poorly; I am almost deaf.
5 ( ) I am completely deaf.

QUESTION 4. BREATHING
1 ( ) I am able to breathe normally, i.e. with no shortness of breath or other breathing difficulty.
2 ( ) I have shortness of breath during heavy work or sports, or when walking briskly on flat ground or slightly uphill.
3 ( ) I have shortness of breath when walking on flat ground at the same speed as others my age.
4 ( ) I get shortness of breath even after light activity, e.g. washing or dressing myself.
5 ( ) I have breathing difficulties almost all the time, even when resting.

QUESTION 5. SLEEPING
1 ( ) I am able to sleep normally, i.e. I have no problems with sleeping.
2 ( ) I have slight problems with sleeping, e.g. difficulty in falling asleep, or sometimes waking at night.
3 ( ) I have moderate problems with sleeping, e.g. disturbed sleep, or feeling I have not slept enough.
4 ( ) I have great problems with sleeping, e.g. having to use sleeping pills often or routinely,or usually waking at night and/or too early in the morning.
5 ( ) I suffer severe sleeplessness, e.g. sleep is almost impossible even with full use of sleeping pills, or staying awake most of the night.

QUESTION 6. EATING
1 ( ) I am able to eat normally, i.e. with no help from others.
2 ( ) I am able to eat by myself with minor difficulty (e.g. slowly, clumsily, shakily, or with special appliances).
3 ( ) I need some help from another person in eating.
4 ( ) I am unable to eat by myself at all, so I must be fed by another person.
5 ( ) I am unable to eat at all, so I am fed either by tube or intravenously.

QUESTION 7. SPEECH
1 ( ) I am able to speak normally, i.e. clearly, audibly and fluently.
2 ( ) I have slight speech difficulties, e.g. occasional fumbling for words, mumbling, or changes of pitch.
3 ( ) I can make myself understood, but my speech is e.g. disjointed, faltering, stuttering or stammering.
4 ( ) Most people have great difficulty understanding my speech.
5 ( ) I can only make myself understood by gestures.

QUESTION 8. EXCRETION
1 ( ) My bladder and bowel work normally and without problems.
2 ( ) I have slight problems with my bladder and/or bowel function, e.g. difficulties with urination, or loose or hard bowels.
3 ( ) I have marked problems with my bladder and/or bowel function, e.g. occasional 'accidents', or severe constipation or diarrhea.
4 ( ) I have serious problems with my bladder and/or bowel function, e.g. routine 'accidents', or need of catheterization or enemas.
5 ( ) I have no control over my bladder and/or bowel function.

QUESTION 9. USUAL ACTIVITIES
1 ( ) I am able to perform my usual activities (e.g. employment, studying, housework, freetime activities) without difficulty.
2 ( ) I am able to perform my usual activities slightly less effectively or with minor difficulty.
3 ( ) I am able to perform my usual activities much less effectively, with considerable difficulty, or not completely.
4 ( ) I can only manage a small proportion of my previously usual activities.
5 ( ) I am unable to manage any of my previously usual activities.

QUESTION 10. MENTAL FUNCTION
1 ( ) I am able to think clearly and logically, and my memory functions well
2 ( ) I have slight difficulties in thinking clearly and logically, or my memory sometimes fails me.
3 ( ) I have marked difficulties in thinking clearly and logically, or my memory is somewhat impaired.
4 ( ) I have great difficulties in thinking clearly and logically, or my memory is seriously impaired.
5 ( ) I am permanently confused and disoriented in place and time.

QUESTION 11. DISCOMFORT AND SYMPTOMS
1 ( ) I have no physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
2 ( ) I have mild physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
3 ( ) I have marked physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
4 ( ) I have severe physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
5 ( ) I have unbearable physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.

QUESTION 12. DEPRESSION
1 ( ) I do not feel at all sad, melancholic or depressed.
2 ( ) I feel slightly sad, melancholic or depressed.
3 ( ) I feel moderately sad, melancholic or depressed.
4 ( ) I feel very sad, melancholic or depressed.
5 ( ) I feel extremely sad, melancholic or depressed.

QUESTION 13. DISTRESS
1 ( ) I do not feel at all anxious, stressed or nervous.
2 ( ) I feel slightly anxious, stressed or nervous.
3 ( ) I feel moderately anxious, stressed or nervous.
4 ( ) I feel very anxious, stressed or nervous.
5 ( ) I feel extremely anxious, stressed or nervous.

QUESTION 14. VITALITY
1 ( ) I feel healthy and energetic.
2 ( ) I feel slightly weary, tired or feeble.
3 ( ) I feel moderately weary, tired or feeble.
4 ( ) I feel very weary, tired or feeble, almost exhausted.
5 ( ) I feel extremely weary, tired or feeble, totally exhausted.

QUESTION 15. SEXUAL ACTIVITY
1 ( ) My state of health has no adverse effect on my sexual activity.
2 ( ) My state of health has a slight effect on my sexual activity.
3 ( ) My state of health has a considerable effect on my sexual activity.
4 ( ) My state of health makes sexual activity almost impossible.
5 ( ) My state of health makes sexual activity impossible.

APPENDIX 4. PHONE INTERVIEW QUESTIONNAIRE, STUDY IV

The patient is diagnosed with 1) MRS 2) CG

FACIAL SYMPTOMS

Have you experienced facial edema? 1) yes 2) no

-during the last year 1) yes 2) no

-during the last month 1) yes 2) no

-which part of the face is affected?

-which side of the face is affected: 1) right 2) left 3) both

-is the symptom 1) recurrent 2) persistent

Have you experienced labial edema? 1) yes 2) no

-during the last yea 1) yes 2) no

-during the last month 1) yes 2) no

-if yes: 1) upper lip 2) lower lip

-which side: 1)right 2) left 3) both

-is the symptom 1) recurrent 2) persistent

Have you experienced edema elsewhere in the body? 1) yes 2) no

-if yes, where?

Have you experienced lesions in the mouth? 1) yes 2) no

-during the last year 1) yes 2) no

-during the last month 1) yes 2) no

-comments: type, location, pain, recurrency, etc

Have you experienced angular cheilitis/ ulceration in the corner of the mouth? 1) yes 2)no

-during the last year 1) yes 2) no

-during the last month 1) yes 2) no

Have you experienced facial palsy? 1) yes 2)no

-if yes: 1) recurrently 2) once

- which side: 1)right 2) left 3) both

-during the last year 1) yes 2) no

-during the last month 1) yes 2) no

- comments: note the side of the palsy at different episodes

In MRS patients, treatment?

In CG patients, treatment?

INTESTINAL SYMPTOMS

Do you have a diagnosed intestinal disease?

1) Crohn´s disease 2) ulcerative colitis 3) coeliac disease 4) other _____ 5) no

Have you experienced intestinal symptoms that have required medical examination? 1) yes 2) no

-comments: type, intensity, etc

Have you experienced diarrhea during the last month? 1) yes 2) no

How many times you excrete in 24 hours?

a. 0-2
b. 3-5
c. 6-8
d. 9 or more

Have you experienced abdominal pain during the last month?

a. Yes, almost daily
b. Yes, every now and then
c. Not at all

Have you had to excrete during the night during the last month?

a. no
b. yes, 1-2 nights a week
c. yes, 3-4 nights a week
d. yes, 5-6 nights a week
e. yes, every night

Have you had blood in the feces during the last month?

a. Yes, every time

b. Yes, occasionally

c. Not at all

d. I don´t know (I never look)

Do you avoid some food items in your diet? 1) yes 2) no

-if yes, which and why?

OTHER

Have you been diagnosed with a chronic condition?

- Asthma 1) yes 2) no
- Cardiovascular disease 1) yes 2) no If yes, which?
- Kidney disease 1) yes 2) no If yes, which?
- Other, which?

Do you have regular medications 1) yes 2) no If yes, which?

Do you have a close relative with

- Melkersson-Rosenthal syndrome 1) yes 2) no If yes, relationship?
- Cheilitis granulomatosa 1) yes 2) no If yes, relationship?
- Crohn´s disease 1) yes 2) no If yes, relationship?
- Ulcerative colitis 1) yes 2) no If yes, relationship?

Other comments:

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