Supplementary Case Reports

Patient 1, a Czech girl, was referred for immunological investigation at the age of eight years. She had no family history of serious immunopathological illness. She has two healthy siblings, a brother (now aged 2) and a sister (now aged 5). Her first episode of oral candidiasis was documented at the age of six months. At the age of one year, she was hospitalized for oral candidiasis. She has since been treated locally and with oral ketokonazole two to three times per year, for the same condition. She has also suffered from recurrent respiratory tract infections, with three documented bouts of pneumonia. Her growth is on the 3rd percentile for her age, despite a midparental growth potential on the 35th percentile. Her weight–to-height proportion was normal. Since the age of five years, she has repeatedly been treated for onychomycosis. She has asthma, treated with inhaled budesonide/formoterol. Her IgG2 levels are low, leading to intermittent intramuscular immunoglobulin treatment. At the time of immunological investigation at the age of eight years, it was noted that the patient was red-haired, with one café au lait spot in the lumbar region, but with no candidiasis either in the mouth or on the nails. Laboratory investigation showed normal IgG, IgA, IgM and IgE levels, but IgG2 and IgG4 were undetectable. The patient had normal levels of anti-*Haemophilus influenzae* B (HIB) and antipneumococcal capsular polysaccharide (PCP) antibodies, and marginally low levels of anti-tetanus toxoid antibodies. Total hemolytic complement (CH50 and AH50 test) levels were normal. The proportion of $CD4^+$ cells was slightly low, at 25% (normal range: 27- 53%), but the absolute numbers of these cells and of the cells of other lymphocyte subpopulations were normal. Overall, 25% of the $CD4^+$ cells expressed CD45RA and 66% expressed CD45RO. Subsequent vaccination with tetanus toxoid and 23-valent pneumococcal polysaccharide vaccine induced a normal antibody response. Lymphocyte proliferation tests showed a slightly impaired response to

stimulation with phytohemagglutinin (PHA) and concanavalin A (ConA). Neutrophil oxidative burst capacity was normal, as shown by the dihydrorhodamine oxidation test. Tests for anti-nuclear, anti-mitochondrial, anti-thyroid gland and anti-smooth muscle antibodies were negative. The patient displayed a slight alteration to liver function enzymes. She was given ketoconazole and cotrimoxazole prophylaxis, which decreased the number of respiratory tract infections; treatment was discontinued after nine months. Approximately six weeks after the discontinuation of treatment, a new episode of pneumonia occurred, accompanied by hypernatremia, hypochloremia, hypoglycemia and hepatopathy. Even after the discontinuation of ketokonazole treatment, mycotic manifestations were only mild, with paronychia of suspected mycotic origin and intermittent mild oral thrush, but five months later, the patient developed bronchitis requiring antibiotic treatment. On the basis of the described electrolyte abnormalities and growth retardation the patient was examined by an endocrinologist. Her growth retardation continued, with a standard deviation score (SDS) of -2.3, her bone age was -2 years, but her insulin-like growth factor 1(IGF1) level was in the reference range. The patient had morning fevers and was tired. She was found to have central hypocorticism with an adrenocorticotropic hormone (ACTH) concentration below 5 ng/l, and a peak cortisol concentration in the ACTH test of 285.5 nmol/l. No antibodies against adrenal and pituitary hormones were detected. As the clinical symptoms did not match those for autoimmune polyendocrinopathy candidiasis-ectodermal (APECED) syndrome, brain magnetic resonance imaging (MRI) was carried out, but the pituitary gland displayed no pathological features. An internal carotid fusiform aneurysm was noted, which was considered stable, with no indication for intervention. One month later, the patient was admitted for meningism, fever, diplopia and headache. Computerized tomography (CT) and MRI showed no signs of acute bleeding. Definitive MRI description showed (in addition to the already detected internal carotid artery aneurysm) multiple fusiform aneurysms in the arteries of the

Willis circle. She died several days later, at the age of nine years, from massive repeated intracranial hemorrhage. Polymerase chain reaction (PCR) for panfungal genes was positive with both CSF and blood, but specific PCR tests for *Candida albicans, C. tropicalis, C. glabrata* and *C. crusei, Aspergillus* sp*., Cryptococcus* sp., and zygomycetes were negative. On post mortem examination, renal abscesses were found and the these sites were the only ones giving positive cultures (for *Candida membranifaciens, Cryptococcus curvatus* and *Trichosporon asahii*). No pathogens grew from brain tissues, aneurysms or peripheral blood.

Patient 2 is now 39 years old and is a Czech woman with mucocutaneous candidiasis and hypothyreosis. She is the mother of Patient 3, whose presentation is similar. Both the father and grandfather of P2 suffered from mycotic infections of the oral cavity and tongue. The family history was otherwise unremarkable. P2 has been followed in our department since 2008, when she was referred, together with her daughter, for immunological analyses. P2 had a long history of onychomycosis, which worsened considerably at the ages of 20 and 31 years. She showed some improvement on systemic antimycotic therapy, but displayed residual, chronic and permanent changes on three fingernails. This patient also has a long history of herpes infections. She has herpes labialis, with flare-ups several times per year, well controlled even with local therapy, but sometimes requiring systemic treatment with acyclovir. However, she also suffers from herpetic keratitis, which has led to chronic changes to the cornea. At the age of 39, the patient's mucocutaneous candidiasis is now permanent and she displays chronic gynecologic mycotic mucosal inflammation. Since 2012, P2 has experienced difficulty swallowing and has been diagnosed with esophageal stricture. A polyresistant *Candida albicans* strain was isolated by culture and the patient was successfully treated with posoconazole for two weeks. She is also being followed by the endocrinology department, for hypothyreosis, which is now stable after

substitution treatment. The patient has permanent hypergammaglobulinemia, with IgG concentrations remaining over 20 g/l for years, and IgA deficiency. She has a low proportion of B cells (between 2.5 and 6%) and her NK cell counts are gradually falling (currently 2%, 0.04 E9/l). The patient has a normal blood count, slightly high levels of IgG antibodies against *Saccharomyces* (ASCA), but no IgA (complete IgA deficiency). The patient is currently in a stable condition, on continual Mycomax treatment, and is awaiting a decision concerning possible esophageal surgery for a stricture that was improved to some extent by posoconazole treatment.

Patient 3 is a 12-year-old Czech girl, the daughter of Patient 2, with a positive family history for mucocutaneous candidiasis. She has been followed in our department since the age of eight years. Her personal perinatal history was completely normal. She began to suffer from chronic angular cheilitis in the first year of life, and has presented more extensive candidiasis of the tongue since the age of five years. At the age of three years, she also developed skin and mucosal inflammation in the genitourinary area, which has since been treated continuously with local preparations. She has been diagnosed with hypothyreosis, which is well controlled by substitution therapy. Unlike her mother, she does not suffer from herpes infections. Laboratory investigations showed normal humoral and cellular immunity, with normal levels of all immunoglobulins and normal lymphocyte subpopulations. However, this patient has extremely high levels of ASCA of both the IgG and IgA isotypes. She is being treated with preventive antimycotic therapy (itraconasole); her hypothyreosis is controlled by substitution treatment and she has a very mild clinical presentation.

Patient 4 is a 48-year-old Hungarian woman with CMC since birth. At the ages of three and four years, she began to suffer from genital and nail candidiasis, respectively. At 11 years of

age, her left kidney was removed because of accidental injury. She underwent tonsillo-adenotomy at 19 years of age, because of recurrent tonsillitis and tonsillo-adenitis. This patient has had recurrent, prolonged episodes of aphthous stomatitis. In addition to CMC, she has had recurrent infections with VZV and HSV (Table 1). In particular, at the age of six years, she presented vesicular lesions on an erythematous base, clustered within two adjacent dermatomas on the left side of the trunk. Shingles was diagnosed on the bases of primary VZV infection (chicken pox) two years earlier, the dermatomal appearance of the clustered lesions, burning pain, intense pruritus and low-grade fever. There were no signs of acute neuritis and the patient did not suffer from postherpetic neuralgia following this episode of acute shingles. Symptomatic treatment was applied and the skin lesions healed three weeks after the onset of symptoms. At 30 years of age, the patient presented another episode of shingles, on the right side of the trunk, with typical vesicular lesions clustered in a 10x8 cm area within dermatomas. Oral acyclovir and treatment resulted in a resolution of the episode in three weeks. The patient developed herpes labialis at the vermilion border of the right angle of the mouth at the age of five years. Between the ages of five and 14 years, vesicular herpetic episodes, during which the vesicles typically grew into ulcers and pustules, occurred 1 to three times per year, but, thereafter, the patient had three to four episodes of HSV infection per year. The lesions typically had a diameter of 0.5-1.0 cm. However, at the age of 21 years, the patient experienced burning, tingling and itching on the right cheek, which was followed by severe pain and the covering, within a period of 12 hours, of an area of 8x5 cm of the skin with grouped, erythematous maculopapules, which progressed to vesicles, pustules, ulcers and crusts. This large surface area of HSV-infected skin healed after three weeks, without scar formation; treatment included oral acyclovir and local antiviral medication. Between the overt herpetic skin infections, the patient experienced burning, tingling and itching of the right labial commissure, without vesicle formation, every three to five weeks.

Aphthous stomatitis began in early infancy and has recurred ever since, at intervals of one to three months. The patient most frequently displays three to six painful ulcers on the tongue, gums and buccal mucosa in the anterior part of the oral cavity. However, she has suffered regularly, at intervals of one to two years, from extremely painful attacks of stomatitis with a sudden onset, difficulty eating and drinking, and fever up to 38.5°C. Vesicles were distributed all over the lips, tonsils, tonsillar pillars, uvula and palate, in addition to the anterior oral cavity. These episodes resolved in two to three weeks on oral acyclovir and symptomatic treatment, indicating that they were indeed due to herpes virus infection. The patient recently underwent esophageal balloon dilatation, which has resulted in sustained improvement and relief of retrosternal pain. She has had five episodes of pneumonia since the age of 10 years, and one episode at the age of 38 years was complicated by the formation of pulmonary abscesses.

Patient 5 is a 17-year-old Hungarian girl, the daughter of Patient 4. She has had recurrent oral candidiasis since infancy, and genital and nail candidiasis since the ages of 12 and 16 years, respectively (Table 1). She has also had recurrent infections with VZV and HSV and recurrent pneumonia (Table 1). She developed two episodes of cutaneous VZV infection affecting the medial surface of the right thigh and both sides of the right knee. The second episode occurred at the age of 14 years and was accompanied by severe pain, burning and itching, painful inguinal lymphadenopathy, local edema and neuralgia. The large skin lesion healed without scar formation after three weeks of parenteral acyclovir treatment. This patient first developed herpes labialis in the left corner of the lips at six years of age, and this condition has since recurred once yearly. However, since the age of 14 years, she has also presented labial herpes in the right corner of the mouth or on the lower labial commissure every other month. In addition, she has had aphthous stomatitis every two to three months since the age of six years.

Patient 6 is a 17-year-old patient of Russian ancestry born after an uncomplicated first pregnancy. She weighed 3000 g and measured 50 cm at birth. Neonatal adaptation was uneventful and the patient was breastfed until the age of five months. At one year of age, she was vaccinated against measles and developed cheesy plaques on the oral mucosal and a loss of appetite. Treatment with local agents resulted in temporary improvement. Frequent episodes of oral candidiasis were observed. The patient was vaccinated against poliomyelitis at the age of 13 months. At one and a half years of age, she had recurrent oral candidiasis, which became increasingly severed. She was treated with fluconazole and bifidus bacteria, resulting in four months of remission. She was treated for severe pleuropneumonia of the left lung at the age of 2.5 years. At 4.5 years of age, she developed onychomycosis of the first and third fingers of the left hand. Bilateral pneumonia was diagnosed at the age of five years. After intravenous treatment with beta-lactam antibiotics, an exacerbation of the oral mucositis was observed and the patient was treated with fluconazole for two weeks and itraconazole for an additional two weeks, followed by a once-weekly itraconazole treatment, which, together, resulted in a mucositis-free period. In addition to recurrent pneumonia, this patient often had purulent infections, including furunculosis, periodontitis and one episode of mandibular osteomyelitis. Esophageal candidiasis was first diagnosed at the age of eight years. At the ages of 10 and 14 years, the patient was treated for lobar pneumonia. Computed tomography at the age of 14 years revealed fibrosis in the 9th and 10th segments of the left lung, and fibrotic stripes in the pleura. Despite regular fluconazole administration, at a therapeutic dose, CMC progressed. Fibrogastroduodenoscopy was performed at the age of 15 years and, 18 cm from the incisors, a narrow esophageal segment was found through which it was not possible to pass an 11 mm endoscope. The mucosa of the proximal esophagus was hyperemic and vulnerable, with multiple white stripes and plaques of 2.5 cm in diameter. Mucosal scraping samples contained neutrophil granulocytes, squamous

epithelial cells and pseudomycelium. After three months of fluconazole treatment, it was possible to pass an 8.2 mm-diameter endoscope along the entire length of the esophagus. The mucosa of the esophagus was moderately hyperemic along its entire length and was covered with a white coating. Contact bleeding was observed in the upper third. Contrast X-ray examination with barium showed that the passage of the suspension was unhindered. In the upper thoracic segment, with tight filling, the maximum fixed width of the lumen was 0.5 to 0.6 cm. No wall elasticity defect was observed in the esophagus. Mucosal folds were thickened (up to 0.4 cm), probably due to edema. The patient was 14 years old when a gradual decrease in the susceptibility of *Candida albicans* to fluconazole was detected, together with a decrease in susceptibility to voriconazole. Treatment with posaconazole was therefore introduced and was well tolerated. The esophageal lumen continued to narrow along its entire length. By this time, it was not possible to eliminate *Candida albicans* from the mucosa despite several courses of posaconazole. No endocrinopathy was found and normal menstruation cycles started at the age of 12 years. This patient has displayed a gradual worsening of anemia and takes regular iron supplements. The patient's history contains several other episodes of disease, including chickenpox, which followed a normal course, and two bouts of cystitis. At the age of 15 years, serum iron concentration was 9.5 mmol/l (normal: 10.7-32.2), unsaturated iron binding capacity was 85.7 mkmol/l (normal: 27.8 - 53.7), total iron binding capacity was 95.2 mmol/l (normal: 38.5 - 85.9).

Patient 7 is a nine-year-old patient born to Russian parents who has suffered from CMC since early infancy. She was the product of her mother's fifth pregnancy. The first three pregnancies were terminated by elective abortion, and the fourth pregnancy resulted in the birth of a girl with congenital malformation, who subsequently died from an undefined chronic illness. This CMC patient was born at term and she weighed 3850 g and measured 55 cm at birth. The perinatal period was uneventful. The patient was vaccinated with BCG and HBV at birth and at one month of age, respectively, and then with DTP and poliovirus vaccine at two months of age.

No further vaccination was carried out, due to severe aphthous stomatitis, which developed into clinically typical thrush. Local glycerol treatment resulted in a transient improvement. The infant initially developed normally with mixed feeding (breastfeeding and formula) but she gradually developed perianal and gluteal candidal dermatitis, which progressed to the skin of the inner thighs, lower abdomen and the waist area. The mother refused local antifungal therapy and preferred her own treatments, on which the patient's condition deteriorated further. The patient often choked when she was breastfed, possibly due to oropharyngeal *Candida* infection. Candidal laryngotracheitis was diagnosed at the age of eight months. Course of oral nystatin treatment resulted in a temporary improvement. By the age of one year, the candidiasis had spread to the neck, face and back, and white, cheesy plaques had spread over the oral mucosa. Fluconazole treatment resulted in clinical improvement. Over the next few years, CMC exacerbation was observed on several occasions and was attenuated by oral antifungal agents. At 3.5 years of age, the patient was diagnosed with a diffuse goiter. At the age of five years, high levels of ALT (343-383 U/l) and AST (250 U/l) were picked up in routine laboratory tests. Serological tests for HAV, HBA and HCV were negative. At the age of six years, the patient underwent fibrogastroduodenoscopy, which showed dispersed white patches throughout the esophagus, in addition to focal hyperemia of the abdominal segment of the esophagus. Samples taken from the esophageal patches contained budding *Candida* yeasts and rod-shaped bacteria. Endocrine evaluation suggested autoimmune thyroiditis and subclinical hypothyreosis and the patient was placed on 50 mg/d L-thyroxine. Over the last two years, this patient has displayed periodic exacerbations of candidal mucositis.

Patient 8 is a 13-year-old Ukrainian boy. He was the product of his mother's third pregnancy, the first two pregnancies having ended in spontaneous abortions. His birth weight and length were 4000 g and 54 cm, respectively, and the neonatal period was uneventful. The

patient's nine-year-old sister and his mother are healthy. Since early infancy, this patient has had recurrent oral candidiasis with aphthous stomatitis and cheilitis, every two to three weeks. Intriguingly, his father and paternal aunt suffer from recurrent labial herpes. His sister had chickenpox when he was 12 but he did not contract the disease. Serologic tests revealed that he had had chickenpox without any detectable clinical manifestation of the disease. The oral candidiasis episodes were accompanied by severe pain and difficulties eating and even drinking fluids. Microbiological cultures from buccal and gingival swabs repeatedly yielded *C. albicans*. Aphthous stomatitis episodes were commonly accompanied by diarrhea. It has been difficult to determine whether this patient has had primary herpes simplex virus infection, because he has had many episodes of aphthous stomatitis, probably due to *Candida* infection. Antifungal treatments have mostly been beneficial, supporting the hypothesis that the stomatitis is caused by *Candida* infection. Occasionally, herpes labialis rash was observed on the lips, in the form of small grouped vesicles, which were then disrupted and developed into a crust. This patient has frequently been diagnosed with candidal skin infections and dermatophytosis since the age of three months. He first presented with onychomycosis at eight months of age, and candidal paronychia at 2.5 years of age. Nail candidiasis affected two or three fingernails on each hand before he reached school age, subsequently becoming rarer, with the last episode of candidal paronychia diagnosed at the age of 10 years. At the age of five years, this patient began to complain of retrosternal pain on swallowing solid food. Chest X ray with contrast enhancement at the age of five years, revealed esophageal narrowing to a diameter of 4 to 5 mm over a length of 48 mm in the middle third of the esophagus. At the age of six years, gastroduodenal fibroscopy was performed with an endoscope of 0.75 cm in diameter. Up to 18 cm from the incisors, the mucosa of the esophagus showed multiple erosions, with fibrin and a stricture about 35 mm long. Mucosal biopsy was performed and histological analysis showed a stratified squamous

epithelium. The esophagus was dilated with air several times. The patient underwent esophageal surgery at the age of 6.6 years, because of persistent vomiting. Following laparoscopy and Nissen fundoplication, the patient could swallow again and stopped vomiting. Fibrogastroduodenoscopy performed each year after surgery revealed white patches and small ulcers in the mucous membrane of the esophagus. Treatment with local nystatin and oral fluconazole, itraconazole and valacyclovir, singly or in combination, gave relief from acute symptoms and pain. At the age of 10 years, it was possible to pass an endoscope 10 mm in diameter freely along the entire length of the esophagus. The lower half of the esophageal mucosa was covered with thin, whitish longitudinal bands. The gastric mucosa was moderately hyperemic and several vascular networks were visible. The patient had recurrent bronchitis and several episodes of bacterial pneumonia, which resolved after the parenteral administration of antibiotics. Recurrent X ray-proven bilateral sinusitis improved on oral β-lactam antibiotics, amoxicillin-clavulanic acid in most cases. The patient has complained of itchy, red eyes. He underwent surgery for phimosis at the age of eight years, without complications.

Patient 9 is now 22 years old and is the first son of healthy Ukrainian parents. He was born at term, after an uncomplicated pregnancy, and weighed 3300 g. At the age of three months, a severe rash occurred, which was treated topically with nystatin. At the ages of seven months and 12 months , chest X rays showed pneumonia, and the patient was treated with ceftriaxone on both occasions. Since the age of 12 months, this patient has suffered from recurrent and persistent cough, but sweat tests ruled out cystic fibrosis. A test for antibody against human immunodeficiency virus was also negative. Oropharyngeal thrush persisted and physical examination revealed onycomycosis and recurrent ophthostomatitis, beginning at the age of one and a half years. Parenteral administration of fluconazole resulted in clinical improvement during

treatment, but mucocutaneous candidiasis recurred after the cessation of therapy. At five years of age, the patient developed diffuse dermatophytosis caused by *Trichophyton* spp. and *Microsporium* spp., which was effectively cured by griseofulvin. In the absence of antifungal prophylaxis, thrush and cutaneous candidiasis relapsed and oral azoles became less effective for the eradication of *Candida*. The patient presented two subsequent episodes of pleuropneumonia, which responded to antibiotic treatment. X rays taken at the ages of eight and 14 years are shown. The patient presented total hair loss by the age of eight years. Gastroduodenoscopy revealed *Candida* esophagitis and esophageal stenosis. Three consecutive gastroduodenoscopies and one bronchoscopy examination revealed diffuse mucosal inflammation, edema and esophageal scar formation. Repeated immunological testing showed smaller than normal numbers of T cells (441-642 cell/mm^3) and B cells (26-41 cell/mm^3). Serum immunoglobulin isotype concentrations were as follows: IgG, 5.1-12.5 g/l; IgA, 0.28-2.3 g/l; IgM, 0.34-1.1 g/l. The patient's lymphocytes responded poorly to mitogens (phytohemagglutinin, pokeweed mitogen, concanavalin A), antigens (tetanus toxoid, purified protein derivative) and stimulation with viruses (herpes simplex virus, influenza B virus) and *Candida*. Serum concentrations of adrenocorticotropic hormone, cortisol, parathyroid hormone, thyroid-stimulating hormone, free thyroxine, testosterone and insulin-like growth factor-1 were in the normal range.