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CHARGE Syndrome and Common Variable Immunodeficiency:

a case report and review of literature

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To the editor

CHARGE syndrome (OMIM 214800) is a rare and complex genetic association of clinical features including Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital and/or urinary tract abnormalities, and Ear abnormalities and deafness. Its estimated prevalence is 1 in 15,000 to 17,000 newborns. Most of the patients have a single aetiology mutation within the chromodomain helicase DNA-binding protein 7 (*CHD7*) gene, located at chromosome 8 (8q12). Chromodomain family proteins are known to play a role in transcription activation and repression by chromatin remodeling, but the exact function of the *CHD7* gene has not yet been ascertained (1). In situ hybridization analysis of *CHD7* during embryonic development demonstrated the expression of this gene in the central nervous system, semicircular canals, and the neural crest, organs affected in CHARGE syndrome. Most of features of CHARGE syndrome could be attributed to disruption of neural crest migration (1). In addition, these patients have frequent infections mainly of the respiratory tract, otitis media and sinusitis, supposed to be due to their anatomical anomalies and neurological dysfunction. Also immunological abnormalities described in some CHARGE syndrome patients (1) may account for the increased susceptibility to infections, and worsen morbidity and mortality.

We describe a full-termed baby born from non-consanguineous healthy parents. At birth, she presented bilateral coloboma, choanal atresia abnormalities of left outer ear, atrioventricular canal, patent foramen ovale and small interatrial defect. FISH analysis did not find any deletion in 22q11.2, but *CHD7* gene mutation by sequencing analysis was detected (heterozygosis c.1153C>T mutation) in exon 2. She was fed by nasogastric tube in the first 2 months due to deglutition disorders, but because of large gastric residuals, Nissen fundoplication was performed and gastrostomy feeding started until the age of 4 years. Choanal atresia and heart defects were surgically treated, heart pacemaker and

acoustic prosthesis placed. In the first years of life, she suffered from recurrent airway infections and thrush and from immune thrombocytopenia when 2 year-old as well.

Growth hormone deficiency (GHD) was diagnosed at the age of 6.4 years (height -4.3 SDS). Treatment with recombinant human growth hormone (rhGH) was started but stopped 10 months later, when a cutaneous swelling on the right gluteus and bilateral axillary lymphadenopathy, occurred. A total body computer tomography showed swollen lymph nodes in axillary site and in mediastinum, some pulmonary round lesions (2-10 mm) and diffuse centrilobular tree-in-bud-opacities in the superior right lobe and in the basal segments of left inferior lobe and numerous enlarged lymph nodes in subdiaphragmatic region (Fig. 1). PET/CT imaging showed hypercaptation (SUV max 4.1) and hypermetabolism in the same areas. Histological study of cutaneous lesion and of an axillary lymph node, displayed T cutaneous pseudo-lymphoma with most cells positive for the follicular T-helper cell marker PD1 (Fig. 2), and a reactive lymphadenitis with no signs of lymphoproliferation, respectively. Bone marrow biopsy was also normal.

When 8-year-old, she presented haemolytic anaemia and thrombocytopenia. Biochemistry showed positive direct (IgG) and indirect (panagglutinant) Coombs test, low serum IgG titer (4.4 g/L) and absolute IgA deficiency (<0,06 g/L) with lack of antibody responses to *tetanus toxoid*, *diphtheria* and *haemophilus influenzae* vaccines, despite she had been immunized. Lymphocyte immunophenotype displayed an increased number of memory T helper CD4+CD45RO+ and memory T cytotoxic CD8+CD45RO+ cells, with low titer of memory B lymphocyte CD19+CD27+/CD19+. Double negative CD3+ TCRαβ + CD4-CD8- Tcells resulted within normal values (table 1). On this basis, Evans syndrome and common variable immunodeficiency (CVID) were diagnosed. Mutation analysis in the gene encoding TACI (*transmembrane activator and calcium modulator and cyclophilin ligand interactor*, *TNFRSF13B*) resulted negative.

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She started immunoglobulin replacement with tapering doses of prednisone, achieving a sustained hematological remission and a progressive reduction of infections.

When 8.2-year-old, hypoparathyroidism was diagnosed and treatment with calcitriol and calcium supplementation started. As the CVID diagnosis, auto-antibodies against endocrine glands (thyroid, ovaries, and adrenals) were assayed and resulted negative.

During follow-up, the patient experienced relapsing immune thrombocytopenia and persistently positive Coombs test, poorly responsive to conventional immunosuppressive treatment.

CHARGE syndrome is a complex association of multiple congenital anomalies which shares many clinical findings with the 22q11.2 deletion syndrome such as cleft palate, cardiac malformations, ear abnormalities, hearing loss, growth deficiency, developmental delay, and renal abnormalities (2). The common features and the wide clinical spectrum of these two syndromes can make initial diagnosis challenging.

At the age of 8 years, our patient presented hypoparathyroidism with severe hypocalcemia, a finding that is typically associated with the 22q11.2 deletion. Significant hypocalcemia, however, was documented in a substantial number of patients with CHARGE syndrome (2). It is not yet clear if hypoparathyroidism is one of the manifestations of CHARGE syndrome since only few patients with CHD7 mutation presented hypoparathyroidism (1).

Patients with CHARGE syndrome may have frequent infections, as in this case, that may be partially explained by anatomical anomalies of the craniofacial region and of upper airway, and neurological dysfunction, resulting in swallowing difficulties and aspiration. The possible contribution of immunological abnormalities to these infections has not been extensively studied even though a wide spectrum of immune defects has been described. Their prevalence is difficult to estimate, because current literature mainly consists of anecdotal cases, most of which not genetically confirmed.

Gennerly *et al.* described 4 patients with *CHD7* mutations and clinical features of CHARGE syndrome. Two patients had a T-B+NK+ form of SCID and two had features of Omenn syndrome (3). Writzl *et al* reported 2 *CHD7* mutation positive patients with significant Tcell lymphopenia, but normal B-cell and NK cell numbers (4). Recently, Jyonouchi *et al.* found low absolute lymphocyte counts in 60% out of 25 children with CHARGE syndrome, and two with T-B+NK+ SCID phenotype. Moreover, in the same series a wide spectrum of humoral immunity was also documented: IgG and IgA deficiency and impaired antibody response to protein antigens (2).

In addition a 6-month-girl with CD4 lymphopenia and colon malakoplakia was described (5). A further case of a patient with features of CHARGE syndrome and Hyper-IgM syndrome was reported (6). Fuentes-Pàez *et al.* published the case of a 5-year-old girl diagnosed with non-classical CHARGE syndrome, Hyper-IgM syndrome and conjunctival MALT lymphoma (7). Chopra *et al.* reported 4 patients with CHARGE syndrome with moderate to severe T-cell lymphopenia associated with thymic hypoplasia or aplasia (8).

An extensive study of the immune system including immunoglobulin levels, peripheral B and T-cell subpopulations, T-receptor excision circle (TREC) analysis, T-cell function and vaccination responses in 24 children with genetically proven CHARGE syndrome, was recently performed. All patients had a history of infections, mainly otitis media and pneumonia (1). Decreased T-cell numbers were found in 12 (50%) patients, mainly caused by reduction in naive mature T-cells, together with low amounts of TRECs. Despite normal peripheral B-cell differentiation and immunoglobulin production, 83% of patients had insufficient antibody titers to one or more vaccinations, specifically to Diphtheria and Haemophilus influenzae type b vaccines.

A Successful cord blood transplantation for a CHARGE syndrome patient with *CHD7* mutation showing DiGeorge sequence was also reported. Markert *et al.* described 14 patients with features of CHARGE syndrome who underwent thymic transplantation for complete DiGeorge syndrome (9).

CVID is a frequent immunological disorder whose hallmarks are hypogammaglobulinemia, impaired antibody response to the immunological stimuli and T-lymphocytes dysfunction (10). Most cases are sporadic, approximately 20% are familial, displaying autosomal dominant or autosomal recessive traits. Mutations in at least 10 genes involved in the development and function of B cells have been associated with CVID. Approximately 10% of affected individuals have mutations in the *TNFRSF13B* gene encoding for a protein which plays a role in the survival and maturation of B cells and in the production of antibodies. The clinical spectrum of CVID includes respiratory infections, gastrointestinal diseases, autoimmune diseases, granulomatous manifestations, and cancer. Granulomatous lesions are usually found in lung, spleen, liver and lymph nodes, but can also be observed in other sites such as skin, bone marrow and eye. This complication is estimated to occur in 8–22 % of CVID patients. In some patients an intense lymphoid infiltration accompanies the granulomata leading to “granulomatous lymphocytic interstitial lung disease” (GLILD), which may be associated with a poor outcome. The granulomas in CVID are usually of noncaseating nature, although caseating granulomas were reported to raise the possibility of an infectious etiology as original trigger. Tissue biopsy may be required not only for diagnosis of granulomas but also to differentiate these lesions from other complications such as lymphoid hyperplasia or lymphoma, which also occur in CVID.

CVID patients with granulomas may exhibit a particular immunological phenotype. They present a significant deficiency of isotype switched memory B cells associated with T cell defects. Deficiency in circulating CD3+CD4+ CD45RA+naïve T cells and expansion of the CD3+CD8+CD57+ compartment were predictive of granulomatous disease.

CT imaging of our child revealed lung bilateral nodules consistent with granulomatous lung disease and no clonal lymphoproliferation disorder was demonstrated by a lymph node biopsy. Moreover a depletion of switched memory B cells and of CD4+ naïve T cells was detected in our patient.

For unclear reasons, granulomatous disease is often found in association with autoimmunity, in particular Evans syndrome, immune thrombocytopenic purpura or autoimmune hemolytic anemia.

Autoimmune manifestations, particularly autoimmune cytopenia, are part of the CVID clinical spectrum reported to be as high as 50%, and in many cases they may precede years the onset of the immunodeficiency. Our girl had her first episode of thrombocytopenia at the age of 2 years. Autoimmune cytopenia relapsed after 6 years when the immunodeficiency was completely unmasked, showing a poor response to conventional treatment.

To the best of our knowledge this is the first case of Charge syndrome with CVID. Although we are unable to propose a possible genetic link, our case confirms and extends the association between CHARGE syndrome and immunological abnormalities. This finding suggest to involve also the immunologists in the care of these patients. Immunological evaluation should be performed as soon as CHARGE syndrome is diagnosed and then carefully screened during follow-up to early reveal the onset of immunological dysregulation and to improve therapeutic and preventive management.

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Figures legend

Fig 1. Total body scan showing swollen lymph nodes, lung nodules and opacities

A. Enlarged lymph nodes are bilaterally present in supra- retroclavicular and axillary (the largest on left of 18 mm) sites. Swollen lymph nodes are highlighted in mediastinum: para and subaortic (axial diameter 5-6 mm), paratracheal (axial diameter 18 mm), hilar and subcarinal (50 x 31 x 15 mm).

B, C. Bilateral numerous lung nodules, with diameter between 2-10 mm, and diffuse centrilobular tree-in-bud opacities, especially in the upper lung lobes, are noted.

D. Lymph nodes are evident in: celiac trunk (11 mm), kidney hilum, para-aortic (22 mm) and along the external iliac artery (11 mm)

Fig. 2 Skin biopsy of right gluteus

2A. Low power magnification of the dermo-hypodermal plurinodular lymphoid infiltrate without evidence of epidermotropism.

2B. Small lymphocytic proliferation admixed with some medium sized atypical cells (arrow) at high power magnification.

2C. Immunohistochemistry for CD3. The infiltrate is represented by CD3 positive T-cells.

2D. Immunohistochemistry for PD1. Most of the cells are positive for the follicular T-helper cell marker PD1.

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Table 1. Immunoglobulins titers, Lymphocyte immunophenotype and IgG specific vaccine antibody responses

IgG	4,4 g/L
IgA	<0.06 g/L
IgM	1,21 g/L
CD3+ (pan T)	71%
CD3+ TCR $\alpha\beta$ + CD4-CD8-	1%
CD3+CD4+ (T helper)	49%
CD3+CD8+ (Suppressor)	20%
Ratio CD4+/CD8+	2,45
CD3+CD4-CD8- (γ / δ +)	2%
CD4-CD8-/CD3+ (α / β +)	0.4%
Naïve T helper (CD45RA+CD4+)	6%
Memory T helper (CD45RO+CD4+)	94%
Naïve cytotoxic T (CD45RA+CD27+CD8+)	9%

Memory cytotoxic T (CD45RO+CD8+)	89%
CD16+CD56+CD3- (NK)	7%
CD19+ (pan B)	19%
Memory B (CD19+CD27+/CD19+)	14%
Switched memory B (CD27+IgD-IgM-)	7%
Plasmablasts (CD24-CD38hi)	12%
Transitionals (CD24hiCD38hi)	n.d.
CD19+CD38-CD21low	10%
Tetanus	0.08 IU/ml
Diphtheria	0.06 IU/ml
Haemophilus Influentiae type b	0.05 mg/l

The table displays increased number of memory T helper CD4+CD45RO+ and memory T cytotoxic CD8+ CD45RO+ cells. Low titer of naïve helper and cytotoxic T cells, of memory B lymphocyte CD19+CD27+/CD19+. Concentration of antibodies to diphtheria or tetanus in IU/ml: a concentration 0.10 IU/ml is considered protective. Concentration of antibodies to Haemophilus influenzae type b in mg/l: a concentration >1.0 mg/l is considered protective.

Fig 1. Total body scan showing swollen lymph nodes, lung nodules and opacities



Fig. 2 Skin biopsy of right gluteus

