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NEIL1 is a candidate gene associated with common variable immunodeficiency in a patient with a chromosome 15q24 deletion

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ABSTRACT

We report the first patient with an interstitial deletion of chromosome 15q24.1-q24.3 associated with common variable immunodeficiency (CVID). The 18-year old female patient's clinical and immunological phenotype was compared with 8 additional previously published patients with chr15q24 deletions. A CGH analysis estimated the deletion to be 3.767 Mb in size (chr15: 74,410,916–78,178,418) and the result was confirmed using qRT-PCR. We defined an immune-related commonly deleted region (ICDR) within the chromosomal band 15q24.2, deleted in all four patients with different forms of antibody deficiencies. Mutations in the 14 genes within this ICDR were not identified in the remaining allele in our patient by WES and gene expression analyses showed haploinsufficiency of all the genes. Among these genes, we consider *Nei Like DNA Glycosylase 1* (*NEIL1*) as a likely candidate gene due to its crucial role in B-cell activation and terminal differentiation.

1. Introduction

The chromosome 15q24 microdeletion (OMIM 613406) syndrome is a rare genetic disorder characterized by intellectual disability, global developmental delay, growth retardation, facial dysmorphism, hypertelorism, joint laxity and genital, skeletal and digital anomalies [1,2]. Deletions involving the 15q24 chromosomal region were first reported in 2007 [3] and >30 cases have been described to date [4–13]. These mainly occur as *de novo* deletions and show variable clinical phenotypes and genotypes, with different breakpoints and sizes of the deletions. The syndrome has been diagnosed from 0 to 29 years of age, with an average around 10 years, and has an incidence in the general population of 1 in 42,000 [2].

The chromosomal region 15q24 is complex and includes five segmental duplication (SD) blocks, also known as Low-Copy Repeat (LCR) clusters. Most of the breakpoints in this region are located in one of the five segmental SD blocks, commonly referred to as the 15q24 A, B, C, D, and E breakpoints [8]. These SD blocks have a high level of sequence homology and may cause non-allelic homologous recombination (NAHR) due to misalignment during meiosis, which can occur between tandemly repeated SD blocks [8]. The size of deletion ranges between 1.7 and 6.1 megabases (Mb) and involves a smallest region of overlap (SRO), spanning a 1.2 Mb region, which is deleted in the majority of the patients and includes several candidate genes, *CYP11A1*, *SEMA7A*, *CPLX3*, *ARID3B*, *STRA6*, *SIN3A* and *CSK*, that may lead to the observed clinical symptoms [1].

Approximately 30% of the patients with the 15q24 microdeletion syndrome show recurrent infections, including upper and lower respiratory tract and ear infections, but the underlying mechanism remains unknown. An immunodeficiency disorder could be the potential cause of the recurrent infections in these patients, but no conclusive evidence of a congenital defect has been found thus far, potentially due to the limited immunologic workup for the cases described to date [1,13].

Here, we describe the first patient with an interstitial deletion of chromosome 15q24.1-q24.3 associated with common variable immunodeficiency (CVID), a primary antibody deficiency that shows a considerable phenotypical and genetic heterogeneity. Several disease causing genes for monogenic forms of CVID have previously been identified, even though these only provide a causative mutation in 2–10% of the CVID patients [14,15].

In order to investigate the molecular basis of the immunodeficiency in this patient, we compared the patient's clinical phenotype and immunological features with eight additional chromosome 15q24 deletion

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patients, previously described [4–11] and for whom partial immunological workup was available. We also performed whole exome sequencing, aiming to identify the candidate gene(s) leading to CVID.

2. Methods

2.1. Clinical description of the patient

Our patient is an 18-year-old female, the second child of non-consanguineous parents. Routine laboratory testing at the time revealed a slightly reduced IgA level, borderline IgM and IgG levels within the reference values (Table 1). Standard and high-resolution chromosomal analysis of the patient showed a normal karyotype. At the age of 6 she developed meningitis, but a viral (i.e. Influenza, RSV, CMV or EBV) or bacterial cause could not be identified and she recovered spontaneously 2–3 weeks later. In the following years, the patient suffered from recurrent ear, lung and sinus infections and chronic cough with mucus secretions. All allergy tests were negative. The patient also presented with recurrent bacterial infections of the upper and lower respiratory tract and the skin. Clinical examination revealed an enlarged spleen (17 cm) and generalized lymphadenopathy. At the age of 11, the patient developed a clinical autoimmune lymphoproliferative syndrome, meeting most of the criteria previously described for ALPS [16]. However, *FAS* or *FASL* mutations could not be identified, nor were there any deleterious mutations in other ALPS associated genes (*CASP10*, *CTLA4*, *PRKCD* and *NRAS*) identified. The patient had feeding difficulties, growth complications (short stature) and neurological abnormalities (hypotonia and ataxia). She was developmentally delayed, with mild dysmorphic features including micrognathia, a large forehead, deep set eyes, thin lips, long philtrum, bifid uvula, submucous cleft and a notched hard palate. The patient had long fingers with bilateral clinodactyly and her fourth toes on both feet bilaterally were overlapping with her fifth toes. She also suffered from skeletal abnormalities, with a poor central tone and a C-shaped spine. At the age of 10, array-CGH analysis revealed a *de novo* interstitial deletion on chromosome 15 [del(15)(q24.1-q24.3)]. Her older brother has mild seasonal allergic conjunctivitis and atopic eczema, but he is otherwise healthy. Both her mother and maternal grandmother have mild psoriasis. Informed consent was obtained from all individuals participating in the study, in agreement with the declaration of Helsinki and the study was approved by the Ethical Committee in Stockholm.

2.2. Immunological description of the patient

The patient was diagnosed with CVID due to low levels of immunoglobulins (Table 1) and, given her frequency of infections, she was substituted with subcutaneous immunoglobulins. An immunological investigation of her parents showed normal immunoglobulin levels (Table 1).

The patient's peripheral blood immune phenotype is summarized in Table 2. Despite the normal B-cell counts, the patient exhibited an abnormal B-cell subpopulation profile and had an increased proportion of naive memory B-cells (IgD^+CD27^-), a reduced proportion of marginal zone B-cells (IgD^+CD27^+) and an almost complete absence of class-switched memory B-cells (IgD^-CD27^+). In addition, an abnormal proportion of immature/activated B-cells (IgM^+CD21^+/IgM^+CD21^-) was observed, compatible with the Freiburg group Ib form of CVID. Antibody responses against pneumococcal polysaccharides and hepatitis-B virus were detectable after vaccination, but the tetanus-specific IgG antibody level was low (1.8 mg/L; nv: 4.9–180 mg/L). The proportion of $CD3^+$ $TCR\alpha/\beta^+$, $CD4^-$, $CD8^-$ (double negative) T-cells was 3.65% of the $CD3^+$ lymphocytes (normal range < 2.5%).

The patient was also thrombocytopenic and neutropenic (Table 2). However, no platelet antibodies or HLA-antibodies were detected. The granulocyte immunofluorescence test (GIFT) showed no antibodies against granulocytes and no aberration was observed using

Table 2
Heamatological parameters from the patient with chr15q24 microdeletion and CVID, in 2007 and 2012. Values marked with stars are outside the normal range. ND: not determined. N/A: values not available.

Parameters	2007	2012	Reference range
Immune phenotype			
Leukocytes ($\times 10^9$/L)	4.7↓	3.9↓	5.0–13.0
Lymphocytes (%)	37	38	45–75 (for age 1 y) 30–55 (for age 7 y)
Lymphocytes ($\times 10^9$/L)	1.75	1.49↓	1.5–6.5
Lymphocyte populations			
$CD3^+$ (T-lymphocytes) (%)	84↑	76	30–78
$CD3^+$ (T-lymphocytes) ($\times 10^9$/L)	1.46	1.13	0.7–4.2
$CD3^+CD4^+$ (T-lymphocytes) (%)	59*	55*	25–48
$CD3^+CD4^+$ (T-lymphocytes) ($\times 10^9$/L)	1.04	0.82	0.3–2.0
$CD3^+CD8^+$ (T-lymphocytes)	19	16	16–32
$CD3^+CD8^+$ (T-lymphocytes) ($\times 10^9$/L)	0.33	0.24↓	0.3–1.8
$CD4^+/CD8^+$ ratio	3.17↑	3.39↑	0.9–1.9
$CD19^+$ (B-lymphocytes) (%)	11	19	10–30
$CD19^+$ (B-lymphocytes) ($\times 10^9$/L)	0.20	0.28	0.2–1.6
$CD16^+CD56^+$ $CD3^-$ (NK-cells) (%)	4↓	5↓	6–27
$CD16^+CD56^+CD3^-$ (NK-cells) ($\times 10^9$/L)	0.06↓	0.08↓	0.09–0.9
T-cell subpopulations			
$CD4^+CD45RA^+RO^-$ (naive) (%)	13↓	13↓	18.2–53.2
$CD4^+CD45RA^-RO^+$ (memory) (%)	69↑	75↑	8.3–27.1
$CCR7^-CD45RA^+CD45RO^-$ (effector helper T-cells)	18↑	12↑	1.1–9.0
$CD8^+CD45RA^+RO^-$ (naive) (%)	61	56	18.6–70.6
$CD8^+CD45RA^-RO^+$ (memory) (%)	12↑	21↑	0.7–5.4
$CCR7^-CD45RA^+CD45RO^-$ (effector cytotoxic T-cells)	27↑	23	4.0–24.0
$CD25^+CD4^+$ $CD127^{low}$ Treg (in % of T-cells)	2↓	5	2.8–6.4
$CD4^+$ HLA-DR^+ (%)	*ND*	3	0.9–4.6
$CD8^+$ HLA-DR^+ (%)	*ND*	4	1.4–21.4
B-cell subpopulations			
IgD^+CD27^- (naive) (%)	85	95↓	53.3–86.0
IgD^+CD27^+ (marginal zone) (%)	12	4	3.3–12.8
IgD^-CD27^+ (switched memory) (%)	2↓	<1↓	4.0–22.1
IgM^+CD21^- (activated) (%)	2↓	26↓	33.2–40.1
IgM^+CD21^+ (%)	96	70	31–88
IgM^-CD38^+ (plasma blasts) (%)	0↓	<1↓	1.2–4.0
$IgM^{++}CD38^{++}$ (transitional) (%)	3	8↑	0.8–6.3
Platelets ($\times 10^9$/L)	NA	89↓	150–400
Neutrophils	NA	0.7↓	2.0–8.0

Table 1
Serum immunoglobulin levels in the chr15q24 microdeletion-CVID patient and her parents.

Parameters (Reference range)	Patient CVID-Chr15del		Mother (2012)	Father (2012)
	9 years (2007)	14 years (2012)		
IgG, g/L	6.3 (6.1–14.5)	3.22↓ (6.1–14.5)	12 (6,7–14,5)	7.95 (6,7–14,5)
IgG1, g/L	5.2 (3.7–9.3)	2.47↓ (3.7–9.3)	NA	NA
IgG2, g/L	0.20↓ (1.0–4.85)	0.29↓ (1.0–4.85)	NA	NA
IgG3, g/L	0.83 (0.22–1.16)	0.49 (0.22–1.16)	NA	NA
IgG4, g/L	<0.01↓ (0.04–1,96)	0.00↓ (0.04–1.96)	NA	NA
IgA, g/L	0.32↓ (0.50–0.07)	0.11↓ (0.70–3.65)	1.96 (0.88–4.50)	1.56 (0.88–4.50)
IgM, g/L	1.5 (0.27–1.50)	0.53 (0.27–2.10)	1.59 (0.27–2.10)	0.67 (0.27–2.10)