The Genetic Heterogeneity of Common Variable Immunodeficiency (CVID)

Vassiliki Lougaris¹, Alessandro Plebani¹

¹Pediatrics Clinic and Institute for Molecular Medicine A. Nocivelli, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, Italy

Abstract

CVID represents the most frequent symptomatic primary humoral immunodeficiency. Clinical presentation includes hypogammaglobulinemia, recurrent infections, autoimmune phenomena and increased lymphoma and cancer risk. While the first cases were reported in the early 50’s, the first genetic cause of CVID was described after 5 decades. After the first description, and also thanks to the advances in the field of biomedical research, several additional genetic causes of CVID have been described. The current genetic landscape of CVID includes numerous genetic alterations that may cause or contribute to the development of CVID, underscoring the complexity and heterogeneity of this disorder.

Keywords: common variable Immunodeficiency, lymphoma, hypogammaglobulinemia, autoimmunity

Introduction

CVID is classically characterized by low immunoglobulin serum levels of at least two isotypes (IgG and/or IgA and/or IgM) with defective antibody response to recall immunizations, in the presence of normal peripheral B cell numbers (in the majority of cases). The clinical manifestations of CVID are variable and may include recurrent infections, mainly of the respiratory and gastrointestinal tract, autoimmune manifestations, splenomegaly and lymphadenopathies, granulomata and increased susceptibility to cancer and lymphomas (1). The age of onset is variable, with a higher prevalence during the second and third decade of life; both sexes are involved in an equal manner. The complex and heterogeneous clinical and immunological phenotype of CVID, has long suggested that the pathogenesis of this disorder may be related to immunological alterations related to different genetic defects. In
the last two decades, the genetic studies in patients with CVID have confirmed this hypothesis, underscoring a significant molecular and immunological complexity in CVID.

**ICOS deficiency**

While the first case of CVID was described in the early 50’s (2), it was only after almost 5 decades that the first genetic cause of CVID was identified. In 2003, biallelic null mutations in ICOS were described in four adult-onset CVID patients resulting in lack of expression of ICOS (Inducible T-cell Costimulator), a T cell receptor that binds ICOS-ligand (ICOSL) expressed on B cells (3). Lymph node analysis from an ICOS deficient patient revealed important alterations in the formation and architecture of the germinal centers (4).

**TACI deficiency**

Soon after the description of human ICOS deficiency in CVID, mutations in TNFRSF13B encoding for TACI, expressed on B cells, were identified in patients affected with CVID (5-6). In a cohort of 162 unrelated CVID patients, Salzer et al identified the homozygous p.C104R, p.S144X mutations in TACI that resulted in lack of TACI expression on B cells, with defective binding to APRIL but not to BAFF, since the expression of the BAFF receptor (BAFF-R) was unaltered. Mutated patients’ B cells failed to class switch upon in vitro stimulation with APRIL and BAFF. Heterozygous variants (p.C104R, p.A181E, p.S194X and p.R202H) were also identified in CVID patients and were reported to be associated with defective antibody production (6). In parallel, a second group identified TNFRSF13B mutations in a small number of patients affected with CVID and SlgAD supporting the hypothesis that CVID and SlgAD may share similar pathogenetic mechanisms. Of note, in both studies, the features of patients’ B cells did not reproduce the phenotype of the mouse model, confirming the existence of important differences between the animal and the human immune systems (5, 7).

Screening of larger cohorts of patients for TACI mutations revealed additional genetic variations (5, 6, 8-11), frequently in the heterozygous state, rendering difficult their interpretation in terms of pathogenicity. In a cohort of 564 CVID patients, the p.C104R monoallelic TACI mutation was present in 4.6% of affected patients and in 0.9% of 675 healthy controls, and resulted associated with an elevated risk for the development of hypogammaglobulinemia, lymphoproliferation and autoimmunity (11). Available data suggest that while biallelic TNFRSF13B variants that abrogate TACI on B cells are responsible for CVID, the role of heterozygous variants is still in debate.

TACI, together with BAFFR and BCMA, are members of a TNF receptor superfamily, that bind to BAFF and APRIL (BAFF-R binds only BAFF). Based on available knockout animal data, and after the identification of TACI mutations in patients with CVID, the candidate gene approach led to further investigation of the above mentioned genes.

**BAFFR deficiency**

Analysis of the TNFRSF13C gene encoding for BAFF-R in 48 patients (12) revealed the presence of three novel variants, all at the heterozygous state: p.P21R, p.G64V and p.H159Y. Of interest, the p.P21R variant was recently shown to alter the polymerization of BAFF-R on the surface of B cells, contributing therefore to the pathogenesis of CVID (13). Recently, biallelic mutations in TNFRSF13C, the gene encoding for BAFF-R, were identified in two siblings with adult-onset CVID and low peripheral B cell counts (14). They both carried a homozygous 24bp in-frame deletion (del89-96) located in exon 2 of the TNFRSF13C gene leading to the lack of BAFF-R on B cell surface. Both patients presented hypogammaglobulinemia (low IgG and IgM, normal IgA serum levels). They also did not mount a T-independent immune response against pneumococcal cell wall polysaccharides.
The role of BAFF, BCMA and APRIL


CD19 deficiency

Following the description of mutations in TACI and BAFFR associated with the pathogenesis of CVID, CD19 deficiency was identified in four patients. Three patients harboured the homozygous deletion 1384del(ga), while one harbourd the homozygous insertion 972ins(a). Both mutations led to a premature stop codon and thus lack of CD19 expression on the B cell surface. CD27+ memory B cells were decreased in affected patients, as typically observed in CVID (17). A fifth patient with CD19 deficiency was identified after the initial description, carrying a compound heterozygous mutation in the gene encoding for CD19 (18). More in detail, mutation analysis of CD19 revealed a mutation in the splice acceptor site of intron 5 (IVS5-1G>T) of the maternal allele, resulting in skipping of exon 6, and a truncated protein product. The paternal allele was disrupted by a gross deletion encompassing at least the ATP2A1, CD19 and NFATC2IP genes (18).

CD20 deficiency

Upon CD19 deficiency, CD20 deficiency was identified in a single patient with hypogammaglobulinemia due to a compound mutation of the noncanonical splice donor sequence of exon 5 of the CD20 gene. The patient showed defective antibody formation upon T-independent antigen stimulation, similarly to what observed in the CD20 knockout mice (19).

CD81 deficiency

CD81 deficiency was subsequently identified in a patient with hypogammaglobulin, severe nephropathy and lack of CD19 expression on B cells was described. The lack of CD19 expression was due to CD81 deficiency, a member of the tetraspanin family that interacts with CD19 and CD21 on the B cell surface, due to a homozygous c.561+1G>A mutation in the CD81 gene resulting in a complete lack of CD81 expression. Memory B cells were reduced. As observed in CD19 deficiency, BCR cross-linking failed to activate properly B cells; on the contrary, T cell defects were not observed (20).

CD21 deficiency

An adult patient with recurrent infections and hypogammaglobulinemia presented with lack of CD21 expression on B cells during immunological work-up. Sequence analysis revealed a biallelic deleterious mutation in the CR2 gene (encoding CD21) (c.1225+1G>C; p.W766X). B cell maturation evaluation showed reduced class-switched memory B cells. In vitro experiments revealed absent co-stimulatory activity of C3d in enhancing suboptimal B-cell receptor stimulation. While vaccination responses to protein antigens were normal, the response to pneumococcal polysaccharide vaccination was moderately impaired (21).

The genetic alterations described so far as associated or causative of CVID were related to receptors expressed on the lymphocyte (B or T) cell surface. Novel genetic defects affecting cytoplasmic proteins have been reported afterwards in patients with clinical and immunological phenotype compatible with CVID.

PRKCD deficiency

Salzer et al, (22) reported on a single patient born to consanguineous parents, that presented with hypogammaglobulinemia, progressive B cell lymphopenia and severe autoimmunity. A homozygous (c.1352+1G>A) mutation affecting
a splice site of PRKCD led to absent expression of the encoded protein. Another case of PRKCD deficiency due to a p.R614W homozygous deleterious mutation was reported in a single patient with chronic, low-grade Epstein-Barr virus infection (23). The patient had chronic lymphadenopathy, splenomegaly, autoantibodies, elevated immunoglobulins and natural killer dysfunction. Interestingly, the homozygous p.G510S homozygous mutation in PRKCD was identified in three patients affected with systemic lupus erythematosus, with B cell defective apoptosis and hyperproliferation (24), suggesting that biallelic mutation in PRKCD may give rise to variable clinical and immunological phenotypes.

PIK3CD and PIK3R1 Deficiency

Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ were recently identified in patients with CVID-like phenotype (APDS-1; Activated phosphoinositide 3-kinase δ syndrome) (25, 26). Affected patients presented various features, including lymphopenia, hypogammaglobulinemia, variable B and T cell maturational defects and, of note, T cell senescence (25, 26). Affected patients present an increased prevalence of B-cell lymphomas (27).

In addition, and shortly after the identification of APDS-1, monoallelic activating mutations in PIK3R1, the gene encoding for the regulatory subunit p85α of PI3K, were identified in patients with a CVID-like phenotype (APDS-2). Affected patients presented recurrent respiratory infections, gut involvement, enlarged lymph nodes and tonsils, normal to elevated IgM with low IgG and IgA serum levels, and variable lymphopenia (28-30). Of note, evaluation of NK cells in both APDS-1 and -2 revealed important functional defects (31, 32), a rather uncommon feature for CVID. Since in both APDS-1 and -2 the underlying genetic defect leads to PI3K hyperactivation, targeted inhibition of this cascade, for example with rapamycin, an mTOR inhibitor, or specific PI3K inhibitors, is under clinical consideration (25, 26, 28, 29).

TWEAK deficiency

Three family members (father, daughter and son) affected with classical CVID were found to carry a rare genetic variant (p.R145C) in the gene encoding for TWEAK leading to TWEAK deficiency. Functional in vitro experiments showed that the mutant protein caused inhibition of BAFF-dependent B-cell survival and proliferation, suggesting thus a pathogenetic role of this mutant for CVID in this family (33).

LRBA deficiency

LRBA (lipopolysaccharide responsive beige-like anchor protein) deficiency was recently identified in patients with CVID and/or autoimmune disorders adding further complexity to the pathogenesis of CVID. The first five CVID patients identified harboured homozygous mutations (p.I2657S, p.R1683X, p.E59X and homozygous deletion including exons 1 and 2) in the gene encoding for LRBA that led to loss of protein expression (34). All patients had early onset hypogammaglobulinemia and severe autoimmune manifestations. Immunological evaluation revealed disturbed B cell development, defective in vitro B cell activation, immunoglobulin secretion and proliferation, and defects in B cell autophagy (34). LRBA deficiency due to a homozygous deletion from exon 1 to exon 30 was recently reported in a single patient with autoimmunity but without hypogammaglobulinemia, underlying that LRBA defects may present with variable immunological phenotypes (35). Considering the complicated clinical course of LRBA deficiency, as underlined by long-term follow-up of relatively numerous affected patients (36), HSCT has been implemented in a small number of cases, with variable results (36, 37). Recent experimental data showed that LRBA deficiency leads to defective CTLA-4 expression on T regulatory cells (37, 38), explaining thus the increased prevalence of autoimmunity in this
disorder. Based on these findings, treatment with abatacept, a fusion protein composed by the Fc of IgG1 and the extracellular domain of CTLA-4, has been shown to be a valid alternative to HSCT for a large part of LRBA deficient patients (39).

**NF-KB1 and NF-KB2 deficiency**

Recently, monooallelic mutations in components of the NF-kB pathway were identified in patients with CVID. Regarding the non-canonical NF-kB pathway, monooallelic germline mutations in NFKB2 were described in a small number of patients affected with early onset hypogammaglobulinemia, recurrent infections, autoimmune features and adren al insufficiency (40). The NFKB2 mutations identified lead to altered processing of p100, and therefore affect p52 nuclear translocation. Upon the initial description of NFKB2 deficiency, additional CVID patients with NFKB2 mutations have been described (41-43). Of interest, and similarly to what observed in APDS-1 and-2, NFKB2 mutated NK cells showed defective cytotoxic activity, a rather unusual feature for CVID (43).

Regarding the canonical pathway, monooallelic mutations in NFKB1 were initially identified in adult-onset CVID patients. Affected patients presented a classical CVID phenotype characterized by hypogammaglobulinemia, recurrent infections and variable autoimmune features. The identified mutations altered the normal processing of p105 and the nuclear localization of p50 (44). Evaluation of B cell maturation showed both early and late B cell developmental alterations in NFKB1 mutated patients (45). Following the identification of this genetic defect, additional cases have been described, broadening the clinical and immunological phenotype that now also includes EBV-driven lymphoproliferation and autoimmune manifestations (46, 47). Of interest, as observed in APDS-1, APDS-2 and NFKB2 deficiency, NFKB1 mutated NK cells revealed functional alterations as well as maturation perturbations (48).

**CTLA-4 deficiency**

A novel genetic form of CVID was recently identified in monooallelic mutations in Cytotoxic T lymphocyte antigen 4 (CTLA-4). CTLA-4 is expressed in activated regulatory T cells and exhibits an inhibitory function on T cell biology. Affected patients present a complex syndrome of immune dysregulation characterized by variable features such hypogammaglobulinemia, lymphopenia, autoimmune cytopenias, lymphoproliferation and granulomas (49, 50), resembling LRBA deficiency (34, 36). They also seem to present an increased risk of gastric cancer, in accordance with recent experimental observations in animal models (51). Targeted treatments such as CTLA4-Ig, may become promising tools for patients affected with CTLA-4 or LRBA deficiency, since LRBA was recently shown to regulate CTLA-4 expression on T regulatory cells (37, 38).

**ADA2 deficiency**

ADA2 deficiency was originally described in patients with vasculitis and early-onset strokes (52). However, patients with a CVID-like phenotype were later identified with biallelic mutations in CECR1, causing ADA2 deficiency (53, 54), with important implications for prognosis and therapeutic strategies (55).

**polymorphisms of MSH5**

Besides the above mentioned genes, genes involved in the DNA repair process have also been implicated in the pathogenesis of CVID. This is the case of MS5, a gene encoded in the central MHC class III region, which plays a critical role in regulating meiotic homologous recombination. Sekine et al. (56) presented evidence that the human MSH5 alleles containing two non-synonymous polymorphisms (p.L85F/p.P786S), may be involved in the pathogenesis of selective IgA deficiency and common variable immune deficiency (CVID).

**RAG1/2 deficiency**

Mutations in RAG1/2 are typically associated with severe combined immunodeficiency (57). However, hypomorphic mutations in these genes
may be associated with a CVID-like phenotype in a limited number of patients (58-60).

**Conclusion**

The scientific advances of the last two decades have allowed to shed light into the genetic alterations that contribute to the pathogenesis of CVID. These important findings underscore the fact that while patients affected with CVID may have a similar immunological phenotype, the underlying genetic causes may be diverse, with important implications in patients’ clinical management, genetic counselling and prognosis.

**Conflict of interest**

The authors declare no conflicts of interests in regard with this study.

**Acknowledgment**

This research received no grant from any financial organizations or funding agency in the public, commercial or not-for-profit sectors. Also, there is no Financial & competing interest disclosure.

**References**

13. Pieper K, Rizzi M, Speletas M, Smulski CR,


46. Schipp C, Nabhani S, Bienemann K, Si-


