



REVIEW ARTICLE

Recent advances in elucidating the genetics of common variable immunodeficiency

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Abstract Common variable immunodeficiency disorders (CVID), a heterogeneous group of inborn errors of immunity, is the most common symptomatic primary immunodeficiency disorder. Patients with CVID have highly variable clinical presentation. With the advent of whole genome sequencing and genome wide association studies (GWAS), there has been a remarkable improvement in understanding the genetics of CVID. This has also helped in understanding the pathogenesis of CVID and has drastically improved the management of these patients. A multi-omics approach integrating the DNA sequencing along with RNA sequencing, proteomics, epigenetic and metabolomics profile is the need of the hour to unravel specific CVID associated disease pathways and novel therapeutic targets. In this review, we elaborate various techniques that have helped in understanding the genetics of CVID.

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Introduction

Inborn errors of immunity, a heterogeneous group of uncommon genetic disorders are characterized by impairment of immune system leading to varied clinical manifestations

such as infections, autoimmunity, immune dysregulation, inflammation and malignancies.¹ Nearly 400 genetic defects have been identified in patients with various PIDs till date.^{1–3} CVID is the most common symptomatic PID with predominant antibody deficiency which manifests in older children and adults.⁴ The prevalence of CVID ranges from 1/10,000 to 1/50,000 in the Middle East and Caucasian population; and is less frequently described in African and Asian population.^{4–6} The age of onset of symptoms is highly variable ranging from childhood to second and third decade of life.^{4,7} There is marked genetic and phenotypic heterogeneity in this disease. Several monogenic forms of CVID have been described and have paralleled rapid advancements and development of high-throughput sequencing

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technologies.^{8–13} However, monogenic genetic variants still constitute only 2–10% of all CVID patients in different cohorts.^{4,5,7} The majority of CVID patients lack a monogenic basis and the disease is probably polygenic in origin in most cases. Understanding the genetic basis of CVID will help in developing personalized treatment approaches for better management of patients. This review attempts to illustrate the importance of omics-based technology and integration of next generation platforms in CVID genetics.

Clinical manifestations of CVID

CVID comprises a heterogeneous group of PIDs that present with decreased to undetectable levels of immunoglobulins and an increased susceptibility to develop infections.^{1,2} A large proportion of patients with CVID also have autoimmune manifestations that may sometimes be the presenting or the sole clinical manifestation of disease. Patients with CVID also have predilection to develop malignancies, commonly lymphomas.¹⁴ In addition, patients with CVID may also present with polyclonal lymphoproliferation (lymphoid interstitial pneumonia, persistent lymphadenopathy, splenomegaly, hepatomegaly), chronic inflammatory disorders like granulomas, and colitis.^{4,14,15} CVID is diagnosed by diminished IgG levels, and either low IgM or IgA levels along with absent or reduced antibody specific responses to infection or vaccine, along with exclusion of secondary forms of hypogammaglobulinemia. The clinical profile of patients with monogenic forms of CVID is different and several defects lead to a unique phenotype,¹ in a given patient it may be extremely difficult based on clinical profile alone to determine the causative gene.

Can the causative gene be identified based on clinical profile of patient and what are the therapeutic implications of identifying the genetic defects in patients with CVID?

Although hypogammaglobulinemia is central to all forms of CVID and intravenous immunoglobulin (IVIg) replacement therapy is the cornerstone of management, specific treatment modalities are now being employed depending on the underlying genetic defect. Identification of the causative gene and its functional significance in patients with CVID may significantly alter the management options from IVIg replacement to hematopoietic stem cell transplant or specific targeted therapy. Clinical manifestations may occasionally suggest an underlying genetic etiology.

Mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (*PIK3CD*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), and phosphatase and tensin homolog (*PTEN*) enzymes lead to a similar clinical phenotype characterized by hypogammaglobulinemia, lymphoproliferation, autoimmunity, and a combined immunodeficiency.¹⁶ *PIK3CD* phosphorylates phosphatidylinositol bisphosphate and activates the downstream signalling cascade. Gain of function mutations result in increased level of rheb GTP (Ras homolog, *mTORC1* binding guanosine triphosphate) that leads to blockage of tuberlin hamartin complex and increased activation of *mTOR*

(mammalian target of rapamycin) thereby causing cell proliferation. *PTEN* and *PIK3R1* act as regulators of this signalling cascade by decreasing the levels of metabolically active phosphatidyl inositol trisphosphate. This leads to dephosphorylation of rheb GTP leading to decreased activation of *mTOR*. Hence, loss of function mutations in *PTEN* or *PIK3R1* lead to a similar clinical profile as is seen in patients with gain of function mutations in *PIK3CD* gene. Patients with activated PI3K delta syndrome (APDS) may have low/normal IgG and IgA with normal to high IgM. The immunoglobulin profile may also suggest a clinical possibility of hyperIgM syndrome. Flow cytometry may reveal increased proportion of senescent T cells. Identification of these defects is very important in patients presenting with CVID phenotype because specific targeted therapies with PI3K δ inhibitors or *mTOR* inhibitors are the treatment of choice¹⁷ (Fig. 1). LRBA (Lipopolysaccharide responsive beige-like anchor protein), and CTLA4 (cytotoxic T-lymphocyte associated protein 4) expression is also closely coordinated as they are present together in the Golgi bodies. Mutations in these genes may cause enteropathy, lymphoproliferation, and autoimmunity in addition to infections. Flow cytometry may reveal decreased expression of CTLA4. Management includes use of CTLA-4 agonists such as abatacept, and belatacept.¹⁸ Hematopoietic stem cell transplantation is also an effective treatment modality in these patients. Mutations in the members of the *TNF* (tumor necrosis factor) receptor superfamily; Transmembrane activator, and CAML interactor (*TACI*), *TNF* receptor superfamily member 13B (*TNFRSF13B*), B-cell activating factor receptor (*BAFF-R*), *TNF* receptor superfamily member 13C (*TNFRSF13C*) resulting in the CVID phenotype have no specific clinical features. However, flow cytometry analysis may suggest decreased expression of these proteins. Mutations in the ATPase H⁺ transporting accessory protein 1 (*ATP6AP1*) gene can also lead to a CVID phenotype, however differentiating features includes an X-linked inheritance, and liver involvement with low copper levels.¹⁹ Mutations in the genes encoding for nuclear factor kappa light chain enhancer of activated B cells (*NF- κ B*), Nuclear factor kappa B subunit 1 (*NFKB1*), and Nuclear factor kappa B subunit 2 (*NFKB2*) have prominent endocrinopathies in addition to other autoimmune manifestations and recurrent sinopulmonary infections. Gain of function mutations in the *NFKB2* may lead to similar manifestations as that of *NFKB1* loss of function mutations because of physiological antagonism. Therapy with calcineurin inhibitors which modulate the *NF κ B* signalling may be employed as a treatment modality.²⁰ Similarly, mutations in other genes associated with the CVID phenotype may have suggestive clinical features such as congenital sideroblastic anemia with hearing loss and auto-inflammation in *TRNT1* (TRNA nucleotidyl transferase 1) defects; neutropenia in *TNF* related weak inducer of apoptosis (*TWEAK*) (*TNF* superfamily member 12; *TNFSF12*) defect²¹, abnormal hair, facies, severe diarrhea with villous atrophy, and possible liver involvement in tetrapeptide repeat domain 37 (*TTC37*) gene defect (trichohematoenteric disease).²² Treatment with *TNF* inhibitors has been recently reported to be efficacious in patients with *TRNT1* defects.²³

Some of these clinical manifestations may guide clinicians regarding an underlying genetic defect. However,

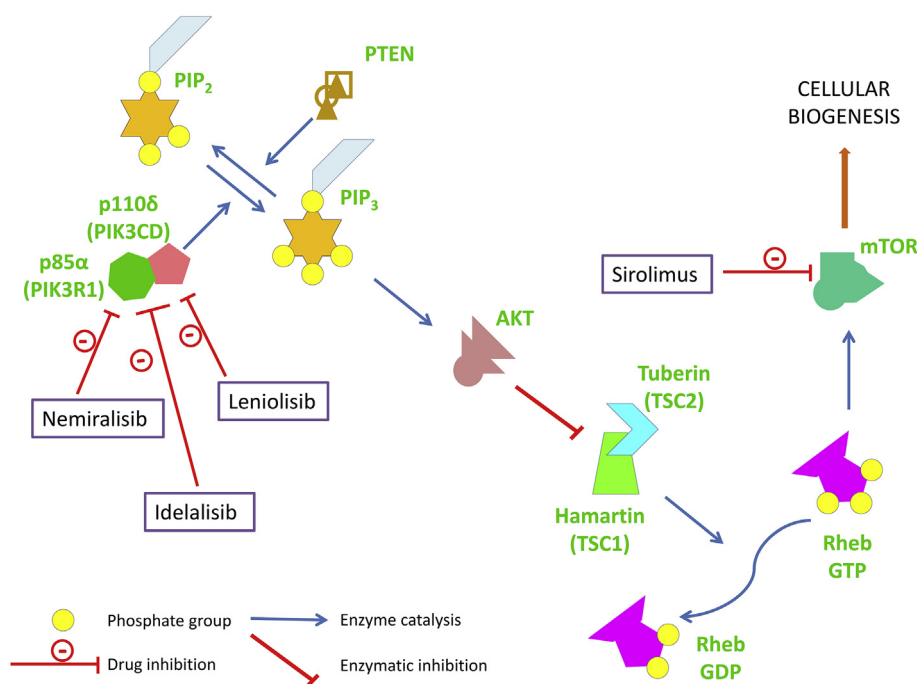


Figure 1 The effect of mutations in Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (*PIK3CD*), Phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), phosphatase and tensin homolog (*PTEN*) genes leading to lymphoproliferation in common variable immunodeficiency disease and their therapeutic modulators. [AKT (Serine/Threonine specific protein kinase); mTOR (Mammalian target of rapamycin); PIP₂ (Plasma membrane intrinsic protein 2); PIP₃ (Plasma membrane intrinsic protein 3); Rheb GTP (Ras homolog, mTORC1 binding guanosine triphosphate); Rheb GDP (Ras homolog, mTORC1 binding guanosine diphosphate); TSC1 (Tuberous Sclerosis Complex 1); TSC2 (Tuberous Sclerosis Complex 2)].

clinical manifestations often overlap and in a given case it may be difficult to identify a candidate gene based only on the available clinical information.

Genetic heterogeneity of CVID has made deciphering the genetic etiologies a challenging task. Over the past decade, CVID has been explored to delineate the monogenic variants, however, besides the genetic variants, epigenetic and environmental factors also play an important role in biology of complex diseases. This needs to be studied extensively to unravel the precise genetic predisposition in these patients. Various genetic defects that have been reported in patients with CVID include mutations in *PIK3CD*, *PIK3R1*, *PTEN*, *CD19* (cluster of differentiation 19), *CD81* (cluster of differentiation 81), *CD20* (cluster of differentiation 20), *CD21* (cluster of differentiation 21), *TAC1* (*TNFRSF13B*), *BAFF* (*TNFRSF13C*), *TWEAK*, *TRNT1*, *TTC37*, *NFKB1*, *NFKB2*, *IKZF1* (IKAROS family Zinc finger 1), *IRF2BP2* (Interferon regulatory factor 2 binding protein 2), and *ATP6AP1* genes.² Illustrating the genetic architecture in patients with CVID phenotype has lead to identification of recessive autosomal inheritance with biallelic mutations in *CD19*, *CD20*, *CD81*, *ICOS* (inducible T cell costimulator), *PRKCD* (protein kinase C delta), and *LRBA*^{24–26} along with autosomal dominant inheritance with monoallelic mutations in *NFKB1*, *NFKB2*, *PIK3CD*, *PIK3R1*,^{27,28} and *IKZF1*.²⁹ Also, the rare hypomorphic mutations associated with severe combined immunodeficiency (SCID) have also been identified in CVID. All of these genes have been reported to play significant roles in immune regulatory pathways such as T-cell signaling, B-cell signaling, and isotype switching.²⁵ However, in majority

(approximately 90%) of CVID patients, no underlying genetic defect has ever been identified till date. Hence, unraveling the genetic basis of sporadic CVID will provide opportunity for patient stratification on the basis of genetic profile and thereby provide insights into therapeutic management of patients.

Technological advancements in elucidating the genetics in CVID

Unlike most PIDs where in a single gene defect has been identified, CVID has a heterogeneous genetic etiology. While several genes have been identified to cause CVID like phenotype in approximately 10% of all patients, no genetic defect has ever been identified in remaining patients.^{5,30} The recent GWAS and next generation sequencing (NGS) platforms have highlighted the alternative theory of polygenic disorders as the underlying cause of CVID pathogenesis. These polygenic determinants are driven by complex gene-gene interactions, incomplete penetrance, and variations in non-coding regions, which are in sharp contrast to monogenic defects with defined genotype-phenotype correlation.^{5,25} Furthermore, it has also been highlighted that the majority of CVID cases are reportedly sporadic which emphasizes the fact that CVID genetics does not follow the classical Mendelian inheritance pattern. Various techniques that have been used in recent time to elucidate the genetic etiology of CVID are as follows:

Next generation screening of CVID

The advent of next generation techniques has revolutionized the identification of genetic basis of diseases and its use has also been extended to patients with CVID.³⁰ The first attempt to elicit pathogenic genetic variants in inherited antibody deficiencies was made in 2010 by Hong-Ying and colleagues who used a customized 300 kb; 148-gene (implicated in immunoglobulin isotype switching and B cell development) re-sequencing Hyper-IgM/CVID chip and reported mutations in *TNFRSF13B*, *AICDA* (activation induced cytidine deaminase), *CD40LG* (cluster of differentiation 40 ligand), and *IKBKG* (inhibitor of nuclear factor kappa B kinase regulatory subunit gamma), along with novel mutations in TNF receptor associated factor 3 interacting protein 2 (*TRAF3IP2*) (rs13190932:C > T and rs33980500:G > A).³¹ This was followed by GWAS study in 363 CVID patients whereby authors quoted the association of CVID pathogenesis and copy number variations (CNVs) with *ADAM* (A Disintegrin and Metalloproteinase) and MHC (Major Histocompatibility Complex) genes.³² Following the initial reports on mutation profile, in 2015 two studies documented decreased diversity in VDJ gene rearrangement, and abnormal complementarity determining region 3 (CDR3) formation which may explain immunodeficiency and increased auto-reactivity in CVID patients.^{33–36} Another study published in 2015 documented *RAG1* (recombination activating 1) mutations in two CVID patients and the group anticipated that the judicious application of NGS will help in elucidating the single gene defects in solving challenging cases.³⁷ The group further emphasized that the early identification of *RAG1* mutations may help in expediting hematopoietic cell transplantation in CVID patients and thereby resorting to preventing translation of autoimmune disease into fatal infections. This was further supported by findings of *RAG1* mutations (c.1871G > A, c.2182T > C and p. H249R) in a CVID patient³⁸ and another study reporting with a novel *RAG1* missense variant (c.1123C > G) and frameshift deletion (c.1430delC, p. F478Sfs*14) along with a known missense mutation (rs199474678) in CVID patient.³⁹ Another study extended the use of next generation technology to illustrate reduction of TCR (T cell receptor) repertoire diversity is driven by reduction of naïve T cells and was found to be associated with reduction in class-switch memory B cells and elevated expression of CD21⁴⁰ B cells.⁴⁰ A novel mutation in *IRF2BP2* gene (c.1652G > A) was identified in three family members of CVID patients which was reported to be linked to monogenic phenotype of CVID and lead to production of mutant protein which was reported to have effect on B-cell differentiation leading to decline in *in vitro* plasmablast production.⁴¹ Another novel heterozygous frameshift mutation was documented in *NFKB1* which lead to a premature stop codon thereby resulting in reduced p105 phosphorylation and decrease p50 protein expression.^{42,43} This loss-of-function mutation attributed to *NFKB1* haplo-insufficiency and has been documented to be related to immunodeficiency. Two other studies reported mutations in CVID patients in *CECR1* (cat eye syndrome chromosome region, candidate 1) gene, which encodes for adenosine deaminase 2 (*ADA2*)⁴⁴ and seventeen probable monoallelic mutations in *STAT3* (signal

transducer and activator of transcription 3), *PIK3CD*, *NFKB1*, *CTLA4*, and *IKZF1* and biallelic mutations in *STXBP2* (syntaxin binding protein 2), and, *LRBA* while screening 50 CVID patients.²⁵ Jan et al (2017) reported several known mutations in *STAT1* (signal transducer and activator of transcription 1), *NLRP3* (NLR family pyrin domain containing 3), *MEFV* (Mediterranean fever), *TNSFR13B*, and novel mutations in *LIG1* (DNA Ligase 1), *MX1* (MX domain like GTPase 1), *FBN1* (Fibrillin-1), *DSG1* (Desmoglein 1), *UNC13D* (Unc-13 homolog D), *TLR1* (Toll like receptor 1), *NBPF15* (NBPF member 15), *FASN* (Fatty acid synthase), *IL1A* (Interleukin 1 alpha), *LAX1* (Lymphocyte transmembrane adaptor 1), *SF3B1* (Splicing factor 3b subunit 1), *CHD7* (Chromodomain helicase DNA binding protein 7), *TUBB1* (Tubulin beta 1 class VI), *ATM* (ATM Serine/Threonine kinase), *TYK2* (Tyrosine kinase 2), *LRRC8A* (Leucine rich repeat containing 8 VRAC subunit A), *PRKCD*, *CFHR5* (Complement factor H related 5), *LRBA*, *EPG5* (Ectopic P-granules autophagy protein 5), *RAG2* (Recombination activating 2), *RAG1*, *NCF2* (Neutrophil cytosolic factor 2), *PTPRC* (Protein tyrosine phosphatase receptor type C), and *MASP2* (Mannan binding lectin serine peptidase 2) in seven CVID patients.⁴⁵ A small subset of CVID patients, have been identified with monogenic defects, however, with introduction of whole genome sequencing and GWAS, polygenic basis of CVID disease etiology has also surfaced.⁴⁶ In a recent study published by Ran et al (2018), three CVID patients were screened using NGS platform which reported with differential mutation profile (*NFKB1* and *LRBA*) in each of the patient.⁴⁷ Two other studies accomplished in 2018 reported genetic mutations in *LRBA*, *ZBTB24* (Zinc finger and BTB domain containing 24), *DNMT3B* (DNA methyltransferase 3 beta), *CTLA4*, *NFKB1*, *PIK3R1*, *PRKCD*, *MAPK8* (Mitogen-activated protein kinase 8), and *DOCK8* (Dedicator of cytokinesis 8).^{48,49} Despite use of next generation technologies for unraveling the underlying genetic defects in CVID patients, different studies have reported differential variants which are attributed to genetic diversity among populations (Table 1). An amalgamation of sequencing data from different cohorts will be useful to identify a panel of genes which are responsible for pre-disposition of patients to CVID phenotype. Hence, in view of recent technological advancements, the genetic screening need to be further elaborated for effective disease management at early stage and targeting these altered genes may present as potential candidate for treatment on therapeutic front.

Transcriptional regulation of CVID

The recent genetic platforms have tried to unravel the novel genetic mutations corroborated with patient's pre-disposition to PIDs. However, enormous data generated from NGS platforms have not been able to uncover the probable causes of PIDs which is in turn attributed to 70% of the human genome transcribed into non coding RNAs. The advent of RNA sequencing surmounted the lacunae as it provides quantitative determination of both coding and non-coding RNAs which will help in characterizing and identifying genetic basis of PIDs. Holm et al (2004) documented the predominance of CCR7^T (C–C chemokine re-

ceptor type 7) cells in subgroup of CVID patients.⁵⁰ In another study conceptualized in 2013 on whole blood transcriptome analysis of CVID patients, the group reported up-regulation of IFN (Interferon) responsive genes manifested pertaining to impaired adaptive immunity which lead to aberrant activation of innate IFN downstream pathway.⁵¹ In another study; RNA sequencing in 20-year-old female patient revealed a novel heterozygous *NLRP12* (NLR family pyrin domain containing 12) mutation along with down-regulation of CCR3 (CC chemokine receptor 3), IFN- γ (Interferon-gamma), and CCR4 (CC chemokine receptor 4) expression.⁵² In another study, van Schouwenburg and colleagues illustrated *TNFRSF13B*, *LRBA*, *NLRP12*, and *TNFRSF13C* variants were associated with up-regulation of *NLRP12*, *ICAM1* (Intercellular adhesion molecule 1), *CD81*, and *PLCG* (Phospholipase C, gamma) upon corroborated analysis of whole genome and RNA sequencing data.⁷ The transcriptome profile of CVID patients still needs to be illustrated extensively to resolve the unanswered dimensions of CVID pathogenesis. In view of limited literature concerning the transcriptome expression of CVID patients, this needs to be further elucidated to unravel the underlying mystery of major portion of the genome transcribed into non-coding RNAs.

Epigenetic dysfunction of CVID

Despite extensive research in CVID patients, epigenome of CVID patients still remains a dimension unexplored. The relevance of epigenetic changes in CVID pathogenesis is illustrated by the role differential DNA methylation plays in the development and activation of B cells.^{53,54} This is attributed to CVID disease etiology which is not associated with a specific genetic defect and varies among patients with identical genetic alterations either because of polygenic defects or environmental factors which influence disease susceptibility in CVID patients. The first evidence of epigenetic pre-disposition of CVID was reported in 2015, when researchers of the Chromatin and Disease Group from the Bellvitge Biomedical Research Institute (IDIBELL) and La Paz Hospital (IDIPAZ) reported epigenetic modifications in identical monozygotic twins discordant for the disease.⁵⁵ The group reported hyper-methylation of *RPS6KB2* (Ribosomal protein S6 kinase beta-2), *BCL2L1* (B-cell lymphoma 2 like 1), *PIK3CD*, *KCNN4* (Potassium calcium-activated channel subfamily N member 4), and *TCF3* (Transcription factor 3) genes, associated with B lymphocytes, led to defective memory cell generation, and aberrant presentation of CVID. In 2015, another report by Rebecca and colleagues documented increase in methylation of *PAX5* (Paired Box 5) enhancer region leading to *PAX5* gene silencing in bone marrow of equine CVID patients.⁵⁶ In another recent study Kienzler and colleagues, emphasized on the role of epigenetic modifications in CVID and its importance in understanding the altered gene expression in pathogenesis of CVID.⁴⁶ The B cells in CVID cells have an impaired knack to up-regulate and de-methylate genes associated with naive to memory B cells transition.^{57,58} In a study published in 2018 insights into cross-talk mechanism between immune activation, gut microbiome and epigenetic alterations in CVID for administration of personalized

medicine based on clinical phenotype and molecular genotype.⁵⁹ Lucia et al (2019) in a recent study suggested that the impaired de-methylation in CVID patients while undergoing transition from the naive to memory B cells in genes of CpG islands has been implicated in B cell signaling (*STAT3*, *FOXO* (Forkhead Box O), *AKT1* (AKT serine/threonine kinase 1), and *NFKB2*), and at GC reaction in *BCL6* (B-cell lymphoma 6), and *AICDA*, these may contribute to the defects reported in terminal B cell of CVID patients.⁶⁰ In view of available scientific evidence, epigenetic treatment may hold as new paradigm of treatment avenues in CVID patient cohort.⁶¹

Advantages and limitations of genetic detection methods

Single gene defects in PIDs were initially identified using Sanger sequencing which involves dye-terminator methodology for DNA fragments which is coupled with capillary electrophoresis. Sanger sequencing till date remains the gold standard for DNA sequencing and is best carried out for known DNA fragments for mutational analysis. However, the major limitation of the technique includes more cost and time along with limited scalability, low throughput and resolution. Later on, sequencing-by-synthesis technique, pyro-sequencing was developed. This technology was based on nucleotide synthesis with release of pyrophosphate. The pyrophosphate is enzymatically converted to ATP, which comes in contact with enzyme luciferase leading to light production and addition of dNTPs individually to the growing DNA molecules. The major challenge encountered with this technique is the addition of new enzyme with every addition of nucleotide, laborious washing of sample between each nucleotide addition and correlation of signal intensity to incorporation of number of bases which is problematic at times for sequencing the homopolymer stretches. The major breakthrough in the sequencing technology, came with 13-years long, Human Genome Project (HGP) which was completed in 2003.^{62,63} With the transition of sequencing technology to development of next generation sequencing based technology (Illumina, Roche 454, Ion Torrent/PGM sequencing, Oxford Nanopore) for rapid amplification of larger stretch of human genome, the sequencing platforms allowed for sequencing with 30 $\times$ coverage or more. NGS allows for massive parallel sequencing, wherein millions of DNA fragments per sample are sequenced with high precision and accuracy. The main advantages of NGS has been the feasibility to sequence whole genome and whole exome which have in turn helped decipher the underlying genetic basis of many diseases which remained unanswered till date. Of the NGS technologies; high throughput sequencing of human genome unravels genetic basis of diseases both in the coding and regulatory pathways. Transcriptome sequencing transitioned from microarray to RNA-seq and have helped decipher the entire transcriptome map associated with disease patients and provides information about the RNA expression profiles. DNA methylation profiling additionally helps study the expression profile of high and low methylated CpG islands intensity to study the role of epigenetic modifications in disease biology. With continuous technological

Table 1 Genetic alterations identified through different next generation platforms in CVID samples. A Disintegrin and Metalloproteinase (ADAM); Activation induced cytidine deaminase (AICDA); Adenosine deaminase 2 (ADA2); AKT serine/threonine kinase 1 (AKT1); ATM Serine/Threonine kinase (ATM); B-cell lymphoma 2 like 1 (BCL2L1); B-cell lymphoma 6 (BCL6); C–C chemokine receptor type 7 (CCR7); CD40 ligand (CD40LG); CD 81 molecule (CD81); Cat eye syndrome chromosome region, candidate 1 (CECR1); Complement factor H related 5 (CFHR5); Chromodomain helicase DNA binding protein 7 (CHD7); Cytotoxic T-lymphocyte associated protein 4 (CTLA4); DNA methyltransferase 3 beta (DNMT3B); Dedicator of cytokinesis 8 (DOCK8); Desmoglein 1 (DSG1); Ectopic P-granules autophagy protein 5 (EPG5); Forkhead Box O(FOXO); Intercellular adhesion molecule 1 (ICAM1); Interferon (IFN); DNA Ligase 1 (LIG1); Fatty acid synthase (FASN); Fibrillin-1 (FBN1); Inhibitor of nuclear factor kappa B kinase regulatory subunit gamma (IKBK); IKAROS family zinc finger 1 (IKZF1); Interleukin 1 alpha (IL1A); Interferon regulatory factor 2 binding protein 2 (IRF2BP2); Lymphocyte transmembrane adaptor 1 (LAX1); Lipopolysaccharide-responsive beige-like anchor protein (LRBA); Leucine rich repeat containing 8 VRAC subunit A (LRRC8A); Mitogen-activated protein kinase 8 (MAPK8); Mannan binding lectin serine peptidase 2 (MASP2); Mediterranean fever (MEFV); MX domain like GTPase 1 (MX1); NBPF member 15 (NBPF15); Neutrophil cytosolic factor 2 (NCF2); Nuclear factor kappa B subunit 1 (NFKB1); Nuclear factor kappa B subunit 2 (NFKB2); NLR family pyrin domain containing 3 (NLRP3); NLR family pyrin domain containing 12 (NLRP12); Paired Box 5 (PAX5); Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD); Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1); Phospholipase C, gamma (PLCG); Protein kinase C delta (PRKCD); Protein tyrosine phosphatase receptor type C (PTPRC); Recombination activating 1 (RAG1); Recombination activating 2 (RAG2); Retinoblastoma associated (RBA); Potassium calcium-activated channel subfamily N member 4 (KCNK4); Ribosomal protein S6 kinase beta-2 (RPS6KB2); Splicing factor 3b subunit 1 (SF3B1); Signal transducer and activator of transcription 1 (STAT1); Signal transducer and activator of transcription 3 (STAT3); Syntaxin binding protein 2 (STXBP2); Transcription factor 3 (TCF3); Toll like receptor 1 (TLR1); TNF receptor superfamily member 13B (TNFRSF13B); TRAF3 interacting protein 2 (TRAF3IP2); Tubulin beta 1 class VI (TUBB1); Tyrosine kinase 2 (TYK2); Unc-13 homolog D (UNC13D); Zinc finger and BTB domain containing 24 (ZBTB24).

Technique	Patients Enrolled in study	Genes Alterations/Mutation	Year	Reference
Next Generation Sequencing Technology				
Hyper-IgM/CVID custom 148 gene Re-sequencing chip	34 CVID patients	<i>TNFRSF13B, AICDA, CD40LG, IKBKG, TRAF3IP2</i>	2010	31
Genome-wide SNP genotyping (InfiniumII HumanHap610 BeadChip)	363 CVID patients	CVID association with ADAM and MHC genes.	2011	32
IGH rearrangements (Roche 454 sequencing)	18 CVID patients	VDJ rearrangement and abnormal formation of complementarity determining region 3 (CDR3)	2015	33
High-throughput DNA sequencing of immunoglobulin heavy chain gene rearrangements (Roche 454 DNA sequencing)	93 CVID patients	VDJ rearrangement and abnormal formation of complementarity determining region 3 (CDR3)	2015	35
Whole exome sequencing (1st patient) and Targeted Gene Panel (2nd patient)	2 CVID patients	1st Patient: <i>RAG1</i> (c256_257delAA, c1835A > G) 2nd Patient: <i>RAG1</i> (c.1566G > T, c.2689C > T)	2015	37
Targeted PID gene sequencing (Ion PGM)	1CVID patient	<i>RAG1</i> mutation (c.1871G > A, c.2182T > C and p.H249R)	2016	38
Next-generation sequencing of TCR repertoire (ClonoSIGHT platform (Sequentia))	42 CVID patients	Decrease in TCR repertoire diversity, naive T cells, and thymic volume was consistent with orthogonal evidence supporting thymic failure in CVID patients.	2016	40
Whole exome sequencing	A family with 3 affected individuals with CVID and unaffected family members	Novel mutation <i>IRF2BP2</i> (c.1652G > A:p(S551N))	2016	41
Targeted exome sequencing (Illumina HiSeq 3000)	1 patient	Novel frameshift mutation in <i>NFKB1</i> c.491delG (p.G165A*31)	2016	42
Whole exome sequencing	5 family members of CVID patient	Two Heterozygous mutation in <i>CECR1</i> (encoding <i>ADA2</i>) rs77563738 and novel Chr22:17,684,478 A > C)	2016	44
Whole exome sequencing (IlluminaHiSeq 2500)	50 CVID patients	Monoallelic mutations (<i>NFKB1</i> , <i>STAT3</i> , <i>CTLA4</i> , <i>PIK3CD</i> and <i>IKZF1</i>) Biallelic mutations (<i>LRBA</i> and <i>STXBP2</i>)	2016	25

(continued on next page)

Table 1 (continued)

Technique	Patients Enrolled in study	Genes Alterations/Mutation	Year	Reference
Targeted Sequencing (MiSeq (Illumina))	1 patient	<i>RAG1</i> : Three mutations. Two Novel: A missense variant (c.1123C > G) and frameshift deletion (c.1430delC, p.F478Sfs*14) and a known missense mutation (c.1420C > T, rs199474678)	2017	³⁹
Whole exome sequencing (HiSeq 2000 or NextSeq 500 (Illumina))	7 CVID patients	<i>STAT1</i> , <i>NLRP3</i> , <i>MEFV</i> , <i>TNSFR13B</i> , <i>LIG1</i> , <i>MX1</i> , <i>FBN1</i> , <i>DSG1</i> , <i>UNC13D</i> , <i>TLR1</i> , <i>NBPF15</i> , <i>FASN</i> , <i>IL1A</i> , <i>LAX1</i> , <i>SF3B1</i> , <i>CHD7</i> , <i>TUBB1</i> , <i>ATM</i> , <i>TYK2</i> , <i>LRRC8A</i> , <i>PRKDC</i> , <i>CFHR5</i> , <i>LRBA</i> , <i>EPG5</i> , <i>RAG2</i> , <i>RAG1</i> , <i>NCF2</i> , <i>PTPRC</i> and <i>MASP2</i> mutations.	2017	⁴⁵
TCR β High-throughput sequencing (Adaptive Biotechnologies)	44 CVID patients	CVID TCRs had reduced junctional diversity and CVID CD3 sequence had increased clonality.	2017	³⁴
Targeted Sequencing (MiSeq (Illumina))	1 patient	<i>NFKB1</i> frameshift mutation (c.1149delT, p.Gly384Glu * 48)	2018	⁴³
TCR Repertoire sequencing (Roche 454 sequencing)	33 CVID patients	CVID patients had defective V(D)J recombination along with somatic Hyper-mutation (SHM).	2018	³⁶
Whole exome sequencing (IlluminaHiSeq 4000)	3 CVID patients	1st patient: <i>RBA</i> (c.8436G > C and c.4089A > T) and <i>TNFRSF13B</i> (c.226G > A) 2nd patient: <i>LRBA</i> (c.3764G > C) 3rd patient: <i>LRBA</i> (c.5084T > C) and <i>NFKB1</i> (c.666dupG).	2018	⁴⁷
Whole exome sequencing (IlluminaHiSeq 2000)	36 CVID patients	<i>LRBA</i> , <i>CTLA4</i> , <i>NFKB1</i> , <i>PIK3R1</i> , <i>PRKCD</i> , <i>MAPK8</i> and <i>DOCK8</i>	2018	⁴⁸
Whole exome sequencing	550 patients (HlgM, CVID and Agammaglobulinemia)	<i>LRBA</i> , <i>ZBTB24</i> and <i>DNMT3B</i> mutations in CVID	2018	⁴⁹
Transcriptome Expression				
GeneChip Human Genome U133A Array (Affymetrix)	23 CVID patients	Enhanced cytotoxic effector functions, Predominance of CCR7 T cells, and Antigen activated T cells	2004	⁵⁰
HT-12 V4 BeadChip (Illumina)	91 CVID patients	Up-regulation of IFN responsive genes.	2013	⁵¹
Whole transcriptome sequencing (IlluminaHiSeq 2000)	1 patient	<i>NLRP12</i> (Heterozygous mutations) encoding NALP12 protein (p.H304Y and p.A629D)	2014	⁵²
RNA sequencing (IlluminaHiSeq 2500)	3 CVID patients	<i>TNFRSF13B</i> , <i>LRBA</i> , <i>NLRP12</i> and <i>TNFRSF13C</i> variants and up-regulation of <i>NLRP12</i> , <i>ICAM1</i> , <i>CD81</i> and <i>PLCG</i> .	2015	⁷
RNA sequencing (IlluminaHiSeq 2000)	7 equine CVID patients	Down-regulation of pro-B cell differentiation genes specifically <i>PAX5</i>	2015	⁵⁶
Epigenetic Alterations				
High-Throughput DNA methylation and Bisulfite-modified DNA pyrosequencing	23 CVID patients	Impaired demethylation in <i>AICDA</i> , <i>BCL6</i> , <i>STAT3</i> , <i>FOXO</i> , <i>AKT1</i> and <i>NFKB2</i>	2019	⁶⁰
High-Throughput DNA methylation	Monozygotic twins discordant for CVID	<i>PIK3CD</i> , <i>BCL2L1</i> , <i>RPS6KB2</i> , <i>TCF3</i> and <i>KCNN4</i>	2015	⁵⁵
Genome wide bisulfite sequencing	Seven equine CVID patients	<i>PAX5</i> gene silencing	2015	⁵⁶

Table 2 Advantages and limitations of different genomic, transcriptomic and proteomics based platforms in Common variable immunodeficiency disease. ELISA, Enzyme-linked immunosorbent assay; SNP, Single nucleotide polymorphism.

Technology	Basic technique	Advantages	Limitations	Ref
Genome-wide Association Studies	> Identify common genetic variants (>5% allele frequency) > Variations in SNPs analyzed throughout genome > Frequent genetic variations considered pointers of disease causing loci	> Hypothesis free approach > Low cost > High resolution > Many loci for single trait concurrently analyzed > Large number of genes can be studied concurrently	> Validation of results required in large data sets and different population > Detects association and not causation > Not predictive and explain less heritability	32,64–67
Sanger Sequencing	> Fluorescent dye-labeled bases > DNA fragments read through capillary electrophoresis	> Long reads (~750bp) > High Sensitivity > High accuracy > Gold Standard > Can be applied to large number of patients	> Low throughput > Time consuming > Detects genetic variation in known region > Cannot detect translocations > Cannot detect copy number changes	37,44,49,68–70
Pyro-sequencing	> Sequencing by synthesis > Chemiluminescent based detection	> More sensitive than Sanger sequencing > % mutated vs. wild-type DNA	> Short Read length > Limited to known hot-spots > Limited accuracy to detect homopolymer changes > Limited scalability	27,35,55,60
Next Generation Sequencing	> Involves array based massive parallel sequencing > Genomic DNA is fragmented and ligated for library preparation followed by amplification and sequencing	> High throughput > Low background noise signal > High sensitivity > Large dynamic range > Nano-grams of starting material required	> Short reads (~100-500bp) > Amplification bias > Massive set-up and infrastructure required > Limited bioinformatics	
Targeted Sequencing		> Detects genetic variations in pre-designed gene of interest > Data is easy to handle	> Not useful where hot-spots or gene of interest is not known	31,38,39,42,43
Whole Exome Sequencing		> Detects genetic variations in protein-coding genome (1% of total genome) > Detects nucleotide variations, small insertions and deletions	> Does not detect genetic variations in non-protein coding genome > Gene expression regulatory regions are not detected	25,37,41,44,45,47,48
Whole Genome Sequencing		> Detects all genetic variations (protein coding and regulatory regions) > Detects all nucleotide variations and genome re-organizations (insertion, deletion, inversion, duplication or translocations)	> Large size of human genome sequencing expensive > Large complex data is generated	30,48

(continued on next page)

Table 2 (continued)

Technology	Basic technique	Advantages	Limitations	Ref
Microarray	➤ Hybridization of complementary sequences via hydrogen bonds to immobilized DNA molecule ➤ Samples are fluorescent dyes	➤ Low cost ➤ High throughput ➤ Well-defined hybridization and analysis pipelines ➤ Easy sample preparation ➤ Large number of samples per run	➤ Analysis based on pre-defined sequence ➤ Limited dynamic range ➤ Non-specific hybridization and High background ➤ Low sensitivity ➤ High variance for low expressed genes ➤ Does not identify splice variants, paralogs and novel transcripts	50,51
RNA-Sequencing	➤ Quantifies and sequence RNA using Next generation technology ➤ Analyze transcriptome of gene expression pattern encoded in RNA	➤ High throughput ➤ High dynamic range ➤ High sensitivity ➤ Low background noise signal ➤ No hybridization ➤ Detects alternative spliced sites, paralogous genes, SNP and non-coding RNAs identification	➤ Protocols not fully optimized ➤ High power computing facilities required ➤ High set-up and run costs ➤ Complex computational analysis ➤ Complex analysis of splice variants	7,52,56
Epigenome profiling	➤ Quantifies DNA methylation at multiple CpG sites	➤ Sodium bisulfite treatment – Gold Standard ➤ Detects gene expression in regions with high and low CpG density ➤ Low cost	➤ Not every methylated region can be captured with affinity enrichment technique ➤ Sensitive to CpG density and copy numbers ➤ Does not identify 5 mC sites ➤ No absolute quantification of methylation levels	55,56,60
Fourier-transform infrared (FTIR) spectroscopy	➤ Monitor biochemical changes on the basis of spectral features which reflect chemical and molecular composition	➤ Rapid ➤ Inexpensive ➤ Non-invasive technique	➤ High noise ➤ Complex data ➤ High end computational methods (chemometrics) required for data analysis	71

advancements the cost incurred to sequence whole exome/genome or transcriptome have drastically been reduced. However, the major limitation of the advanced technologies is the huge amount of data generated from NGS runs with massive parallel sequencing of which data analysis is complex and need high end computational back-up and set-up for bioinformatics analysis. The advantages and limitations of various sequencing technologies (Sanger sequencing, Pyro-sequencing) and NGS platforms (whole exome sequencing, whole genome sequencing, Transcriptome profiling, epigenome profiling and proteome profiling) have been detailed in Table 2. Although, NGS is

cost effective and fast in comparison to first generation sequencing technologies, it still remains expensive for small scale laboratories and individuals. The small read lengths leads to highly repetitive sequences which is a major shortcoming. Further, NGS data is complex, time consuming and need good bioinformatics expertise to comprehend the huge data generated. In view of these limitations, third generation sequencing technologies have been instated to address the gap between NGS and conventional sequencing technologies which involves direct sequencing of single molecules with long read lengths, low cost and time whereby maintaining quality of genome assembly.

Future perspective

The management of CVID patients has transitioned from the initial era of clinical diagnosis to molecular diagnosis. However, despite the technical advancements the precise genetic basis of CVID still remains enigmatic. The genetic diversity among different patient cohorts of diverse ethnic background is another challenge in CVID genetics. The advancements in the field of next generation has brought has many steps closer to treat and manage CVID patients; which remained enigmatic and static over the past decade. The multi-omics approach integrating the findings from NGS platform along with expression profiling, proteomics, metabolomics and methylation patterns concurrently will enhance our understanding of the underlying complexities of CVID genetics. The strength of coalescing complementary technologies together will help comprehend the CVID genetic architecture, which still remains an unanswered paradox. The complex challenges of CVID need to be deciphered for individualization and moderation of therapeutic treatments on individual basis.

Conclusion

The heterogeneous paradigm of CVID still remains a dimension to be explored. Recently, NGS has been actively deployed to comprehend the pathogenic mutations responsible for CVID clinical manifestations. However, pertaining to CVID disease heterogeneity, studies have reported different genetic profile in different cohort of CVID patients which is attributed to ethnic diversity, different risk factors and diverse epigenetic profile. Despite the recent studies to explore the genetic basis of CVID, the scope of translation of genome-wide association studies in patient care is limited in view of small sample size pertaining to relatively low prevalence of CVID in comparison to other primary immunodeficiency disorders. Thus, the available scientific evidence warrants an insight into multi-omics approach which would conjugate next generation sequencing technology, gene expression studies, epigenetics, proteomics and metabolomics together, with an aim to unleash the underlying molecular complexities in CVID pathogenesis. The implementations of next generation techniques to elucidate genetic alterations in health care have helped us realize the potential of precision medicine. The dawn of NGS and 'omic' technologies in molecular research has transformed the perception of looking through genetic and epigenetic diaspora of disease etiology and its associated underlying pathogenesis. Hence, personalized treatment based on phenotypic and genotypic alterations is the need of the hour for CVID patients. In summary, sustained efforts of multi-omics on integrative platform will pave the way ahead for better clinical characterization of CVID which can be translated into therapeutic targets for precision medicine.

Conflict of interest

The authors declare no conflict of interest.

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Authorship

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