Acquired Pure Red Cell Aplastic in one of the Monozygotic Twins with Common Variable Immunodeficiency

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Case report

Keywords: Common variable immunodeficiency, pure red cell aplastic, monozygotic twins, VDR, NHEJ1, Whole exome sequencing

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-740295/v1>

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Abstract

Background

Common variable immunodeficiency (CVID) is the most prevalent primary immune defects in adults. It has become clear that most cases of CVID have a polygenic rather than a monogenic inheritance. CVID patients are prone to recurrent infection, and an increased incidence of certain autoimmune and neoplastic disorders. Monozygotic twins share identical genetic basis and may serve as a powerful model for study on genetic defects.

Case presentation

Here, we report a case of monozygotic twins were diagnosed with CVID at their 30s'. They featured a partly similar profile of clinical manifestations, including severe, recurring infections and bone pain. Interestingly, only the elder brother developed pure red cell aplastic (PRCA), and relieved after 5-month's treatment with 100 mg/d of cyclosporine treatment. Whole-exome sequencing (WES) was utilized to investigate genetic defects.

Conclusions

The results suggest that a combination effect of deleterious variations maybe the cause, such as VDR, NHEJ1, DOCK5, NOD2 and C3, which were predicted by bioinformatics analysis. The potential combinatorial effects in CVID is inferred from their roles in T and B cell signal pathways activation.

1. Background

Common variable immunodeficiency disorder (CVID) is one of the most prevalent primary immunodeficiencies with an important morbidity and high number of medical encounters. Majority of patients are predisposed to recurrent and severe infections as a result of immune system failure. A significant minority suffer from autoimmune disease, sarcoid-like granuloma or malignancy [1]. The reported prevalence is about 1:50,000–1:20,000. Onset can vary from 4 years old to late adulthood [2]. Mortality rate in the first 10 years after diagnosis ranges from 10%-35% in CVID patients [3]. The current CVID diagnostic criteria were developed by International Consensus Document (ICON) in 2016, as follows: the IgG level is repeatedly lower than local reference range; IgA or IgM level also is low; poor response to at least 1 type of antigen; other causes of hypogammaglobulinemia have been excluded [4].

Most of CVID cases occur sporadically, about 5%-25% of patients have a familial tendency, of which most demonstrate an autosomal dominant inheritance [5]. So far, monogenic causes have been identified in 2–10% of affected individuals with both autosomal recessive and dominant inherited, PIK3CD and LRBA gene mutations comprise approximately half of published cases, while more than 30 additional genes have also been reported. It has been recently suggested that, apart from rare monogenic forms, CVID is a complex rather than a Mendelian disease [6]. Whole exome sequencing (WES) is a potential effective tool

to help discover genetic defects, which targets protein-coding sequences accounting for 1% of the whole genome, but reported to harbor 85% of disease-causing variants [7]. WES is commonly used to explore the pathogenic genes of CVID [8, 9].

Here we describe the phenotypes of a twin with CVID. Extensive phenotypic profile of circulating B and T-cell subsets were obtained by flow-cytometry at diagnosis and during several years follow-up. WES allowed us to identify a combination of variants with putative impact in the immune system and support a polygenic basis for the CVID phenotype and their concordant immune evolution.

2. Case Presentation

2.1 Patient samples

Case 1 (proband): a 30-year-old male with a history of 7-year recurrent chronic watery diarrhea, 1-year lower limb weakness and back pain. The result of bone mineral density (BMD) test suggested severe osteoporosis with increased serum alkaline phosphatases (ALP) and parathyroid hormone (PTH). Multiple times of colonoscopy examination indicated that chronic mucosal inflammation and interstitial edema, lymphoid proliferation in the submucosa and multiple lymphoid follicles, no more obvious abnormality was found. Low levels of all immunoglobulin types were determined by nephelometry. Peripheral blood flow cytometry analysis revealed a low proportion of CD19 + cells and CD4+/CD8 + ratio. In January 2017, he suffered with acute severe anemia with normal level of mean corpuscular volume. Reticulocyte count is 5000/ μ l (< 10000/ μ l). Bone marrow evaluation of the patient showed the nucleated erythrocyte was only 1.5% (< 5%) with normal granulocytic and megakaryocytic line age, and early erythroblast was observable as shown in Fig. 1b. Cytogenetic evaluation showed normal male karyotype. Flow cytometry revealed no evidence of a lymphoproliferative disorder and other hematology diseases. Chest computed tomography scan ruled out the presence of thymoma, and there were no evidence of active HIV or viral hepatitis or other viral infection. Autoimmune antibodies were negative. Therefore, the diagnosis of acquired PRCA was made. Hemoglobin recovered after 5-month's treatment with low dose (100 mg/d) of cyclosporine. And then, the maintenance treatment lasted one year.

Case 2: the proband's monozygotic twin brother, who also suffers recurrent respiratory infection and occasional diarrhea for several years. He developed into intermittent claudication due to bone pain in lower limbs since 2016. Symptoms gradually worsen and eventually lead to inability to walk. BMD test suggested severe osteoporosis with increased serum ALP and PTH, low levels of serum immunoglobulin and CD19 + cells proportion.

The twins have no protective titers of antibody to Hepatitis B virus, cowpox vaccines. Intravenous immunoglobulin replacement and oral vitamin D significantly relieved the symptoms of infection and bone pain in the twins. Their clinical characteristics are shown in Table 1 and both met the criteria for CVID [4]. Except an uncle (II:2) of the twins had leukemia, all of the members of their family, including

their parents, were CVID-symptoms free and had normal immunoglobulin level. Father's BMD test is normal. But mother's BMD is lower than peers. The pedigree of the family is shown in Fig. 1a.

Table 1
Clinical features of the twins

Values	II:1 (proband)	III:2	Normal range
Immunoglobulins, g/L			
IgG	1.88	2.28	7.2–16.8
IgA	0.26	0.14	0.82–4.53
IgM	0.18	0.25	0.46–3.04
Lymphocytes, $\times 10^6$ /L	1,256	1,420	760–3,800
Lymphocyte subsets, %			
CD19+	0.1	1.2	4–17
CD4+	16.9	34.64	41–51
CD8+	36.1	37.59	23–33
CD4+/CD8+	0.47	0.92	0.71–2.78
NK	44.6	19.57	5–27
Complement			
C3, g/L	0.29	0.62	0.6–1.5
C4, g/L	0.12	0.19	1.3–3.7
C1q, g/L	0.15	0.13	0.197–0.04
Sedimentation, mm/h	2	3	0–15
C-reactive protein, mg/L	3.45	9.91	0–8
SGOT, IU/L	39	32	13–40
SGPT, IU/L	51	31	0–75
ALP, IU/L	263	548	40–150
Creatinine, μ mol/L	33	42	45–104
Albumin, g/L	32.4	41.8	34–54
Globulin, g/L	10.1	15.9	20–30
Glucose, mmol/L	4.3	4.8	4.1–5.9
Calcium, mmol/L	1.29	1.55	2.03–2.54
Magnesium, mmol/L	0.61	0.73	0.67–1.04

Values	Ⅱ:1 (proband)	Ⅲ:2	Normal range
Osteocalcin, ng/ml	30.74	54.4	40–100
Parathyroid hormone, pg/ml	85.3	280	< 70
25-OH vit D, nmol/L	< 7.5	48.4	75–375
Bone mineral density (BMD)			
lumbar spine	-4.5	-4.09	> -1.0
femoral neck	-4.2	-4.43	> -1.0

The twins and their unaffected parents were recruited for the experiment. All participants provided written informed consent and the study was approved by the Shanghai Jiaotong University School of Medicine Renji Hospital ethic review board.

2.2 Whole exome sequencing

Genomic DNA of the twins and their parents was isolated from peripheral blood mononuclear cells. Exome library capture was performed with the Agilent SureSelect Human All Exon V6 followed by sequencing on the Illumina HiSeq 2500. Low-quality raw reads with joint sequences and Phred-scaled single nucleotide polymorphisms (SNPs) quality of < Q20 were removed by using Cutadapt software and SolexaQA software respectively. High-quality clean reads were mapped and aligned to the reference human genome (hg19) by using Burrows-Wheeler Aligner (BWA) [10]. Polymerase chain reaction (PCR) repeats were removed by Picard tool. All raw variants were annotated with Sorting Intolerant from Tolerant (SIFT), PolyPhen-2, Mutation-Taster, and Combined Annotation-Dependent Depletion (CADD). If required, the variants are validated by Sanger sequencing and real-time quantitative PCR (RT-qPCR). Primers used in these analyses were as follows: VDR-F, TGGACAACAAGAGCGAACT and VDR-R, TTACAAGCCAGGGAAGGAAG; NHEJ1-F, CGATGGAAGAACTGGAGCAA and NHEJ1-R, TCAACAATGGGCGAAGGAG.

2.3 Bioinformatics analysis

Only nonsynonymous single nucleotide variants (SNVs) were considered. According to the 1000 Genomes database, SNVs with the minor allele frequency (MAF) < 0.01 in the general population were considered to be rare, which were potentially significant variants. Variants with CADD score > 10 were predicted deleterious variations. Afterwards, candidate variants were considered in twins, or in twins and father, or in twins and mother. Then, we focused on the variants tied to immunologic pathways (www.genecards.org). Protein structure of mutated genes were predicted online (www3.cmbi.umcn.nl/hope/).

3. Results And Discussion

3.1 Variants likely associated with CVID

Firstly, subtractive analysis of the variant call files between the affected and unaffected family members was carried out using recessive and dominant models according to the law of Mendelian inheritance. The two models respectively revealed 41 and 9 variants but none were predicted to have a deleterious effect on protein function according to SIFT, PolyPhen-2, Mutation-Taster, or CADD.

Then we filtered out 1845 nonsynonymous SNVs from 3401 variants shared by the twins. Among them, 675 rare variants (MAF < 0.01) were screened out in 1000 Genomes database. 398 candidate variants (heterozygous) were predicted to be deleterious based on criteria with CADD score over than 10. But no previously published monogenic CVID variants were identified. Systematic assessment of bioinformatical predictions, published literature, gene annotation and the clinical and immunological phenotype of CVID, reduced the list to seven candidate variants have been reported to confer susceptibility to the disease or to originate similar phenotypes to CVID. The bioinformatics analysis process is shown in Fig. 1c. Among them, VDR (Vitamin D receptor, c.71C > A), NHEJ1 (DNA non-homologous end-joining factor 1, c.475A > G), DOCK5 (dedicator of cytokinesis-5, c.824A > G) and NOD2 (nucleotide-binding oligomerization domain 2, c.1330C > T and c.1411C > T) were inherited from mother. C3 (complement 3, c.2851C > T and c.1940C > T) were inherited from father (detailed in Table 2).

Table 2
Candidate genes associated with CVID pathogenesis

Gene	Chr	mRNA.refGene	Coding change	Protein change	SIFT/PolyPhen-2/Mutation-Taster/CADD*	From	ACMG
VDR	chr12	NM_001017536: exon2	c.71C > A	p.A24D	D/B/N/10.85	mother	U(PM1 + PM2 + PP2)
NHEJ1	chr2	NM_024782: exon4	c.475A > G	p.M159V	T/B/N/12.56	mother	U(PM2)
DOCK5	chr8	NM_024940: exon9	c.824A > G	p.N275S	T/P/D/16.13	mother	U
NOD2	chr16	NM_001293557: exon3	c.1330C > T	p.R444C	T/B/N/11.12	mother	B(PM1 + BS1 + BS2 + BP4)
NOD2	chr16	NM_022162: exon4	c.1411C > T	p.R471C	T/B/N/11.12	mother	B(PM1 + BS1 + BS2 + BP4)
C3	chr19	NM_000064: exon22	c.2851C > T	p.R951C	D/D/N/20.8	father	U(PP2)
C3	chr19	NM_000064: exon15	c.1940C > T	p.T647M	D/P/N/15.23	father	U(PM2 + PP2)
* Using SIFT, PolyPhen-2, MutationTaster, and CADD to predict deleterious SNVs. SIFT (T, tolerated; D, deleterious); PolyPhen-2 (D, probably damaging; P, possibly damaging; B, benign); Mutation-Taster (D, disease-causing; N, polymorphism); CADD (score > 10 implied deleterious variations); ACMG (U, uncertain significance; B, benign; PM1-6, pathogenic moderate; PP1-5, pathogenic supporting; BS1-4, benign strong; BP1-6, benign supporting.)							

3.2 VDR deficiency

Results of Sanger sequencing identified the heterozygous VDR mutation (chr12-48276554: G/T, NM_001017536: exon2: c.71C > A: p.A24D) in the twins and their mother (Fig. 2a). The expression of VDR gene after mutation was significantly lower than that of father's wild type (Fig. 2b). The p.24 mutation was located at the DNA-binding domain (DBD) (Fig. 2c). The DBD is organized into two zinc-nucleated modules, which called as vitamin D-responsive elements (VDREs). Mutations in DBD would result in defective DNA binding and the most severe clinical phenotypes of vitamin D resistance. Through online prediction, we found that the mutant amino introduces a charge, which can cause repulsion of ligands or other residues with the same charge. The mutant residue is bigger than the wild-type and might lead to bumps. The hydrophobicity of the mutant residue will be lost, either in the core of the protein or on the surface (Fig. 2d).

VDR and Vitamin D play an important role in the innate immune system and modulate Toll-like receptor-related responses [11]. VDR can affect immune information transmission and B cell activation by affecting calcium ions [12]. Ardeniz [13] found that VDR expression in patients with CVID were lower in the peripheral blood mononuclear cells and hair follicles when compared to the healthy group. Apparently, VDR also affects bone metabolism. We speculate that osteoporosis symptoms in the twins are associated with VDR mutations. Their increased serum ALP may confirm the association.

3.3 NHEJ1 deficiency

Heterozygous mutation of NHEJ1 (chr2-220012433: T/C, NM_024782: exon4: c.475A > G, p.M159V) occurred in the twins and their mother (Fig. 2e). The expression of mutant NHEJ1 was significantly lower than that of the father's wild type (Fig. 2f). Analysis by the HOPE software suggested that the mutant residue is smaller than the wild-type (Fig. 2g). This will cause a possible loss of external interactions. The mild mutation should not cause misfolding of the protein, but the extra acquired Valine may lead to greater hydrophobicity and may change the interaction between the protein and the corresponding protein (Fig. 2h).

CVID associated with defective DNA double-strand breaks (DNA-DSBs) repair [14]. NHEJ1 mediates the predominant pathway in DSB repair in mammals, especially during V(D)J recombination. V(D)J recombination defects will block differentiation of T and B cells, then lead to immune deficiency in patients [15]. So, we speculate that the NHEJ1 mutation may be involved in the pathogenesis of the twins.

3.4 NOD2 deficiency

NOD2 has been reported to be a possible disease modifying polymorphism in CVID [16]. NOD2 was initially observed for its role in the inflammatory bowel condition Crohn's disease and NOD2-RIP2/NF- κ B signaling activation [17]. Watery diarrhea is one of the classic hallmarks of Crohn's disease. NF- κ B signaling has a vital role in B and T cell activation and development. Thus, we supposed that NOD2 variant may be related to the CVID twins and the clinical manifestation of diarrhea.

3.5 DOCK5 deficiency

As an atypical guanine nucleotide exchange factor, DOCK5 has been extensively studied in cellular functions. Chen [18] has shown that DOCK5 regulates the peripheral B cell differentiation via controlling the CD19-BTK signaling axis as well as actin reorganization through a DOCK5 knockout mouse model. The variant of c.824A > G is novel and has not been reported before. It may be a harmful change through the prediction of PolyPhen-2, Mutation-Taster and CADD.

3.6 C3 deficiency

The complement system and Toll-like receptors (TLRs) are key elements of innate defense mechanisms [19]. B lymphocytes express both TLRs and complement receptors (CRs) [20]. Simultaneous or sequential engagement of these structures expressed either on the cell membrane or intracellularly, may

fundamentally alter and fine tune activation, antibody and cytokine production of B cells. Activation products of the complement system, particularly C3-derived fragments, play an important role in the regulation of B cell responses by binding to their corresponding receptors [21–23]. In our case, C3 variants (c.2851C > T and c.1940C > T) may be harmful changes through the prediction of SIFT, PolyPhen-2 and CADD.

3.7 Acquired pure red cell aplasia

CVID is prone to autoimmune thrombocytopenia, hemolytic anemia and other hematological disorders [24]. As for immunosuppressive agent, cyclosporine shows effective to granulomatous diseases [25] and non-infection diarrhea in CVID patients by suppressing abnormal activation of B cells and T cells [26]. Cyclosporine also has a rapid control effect on our proband regarded as PRCA associated CVID. After 5-month's treatment with cyclosporine, the patient was relieved. This is different from the case Sklarz T. [27] reported that a female was diagnosed with CVID at her 12 years' old. When she was 48 years' old, she was notable for a low reticulocyte count and a hypocellular marrow with an absence of erythroid precursors, consistent with acquired red cell aplasia. However, she was treated with cyclosporine without response and eventually progressed to aplastic anemia at her 50's. The pathogenesis of PRCA happened in CVID is unrevealed, but the phenomenon implies that lack of antibody responses may lead to aberrant cytotoxic T lymphocyte (CTL) responses with cross-reactivity directed to red cell precursors [28].

4. Conclusion And Prospect

With the success of new sequencing technologies, and in particular of WES in unraveling the molecular mechanisms of many rare syndromes, rare diseases such as CVID that do not completely fit with a Mendelian model represent a new challenge for medical genomics. Monozygotic twins are commonly considered to have a similar inherent genetic background and provide a powerful model to dissect the relative contributions of heritable and non-heritable variables to the establishment of clinical and immunological profiles. Silva SL [9] reported a pair of monozygotic twins with CVID have strikingly similar clinical and immune profile associated with a polygenic burden, despite exposure to different living conditions. However, the clinical manifestations of monozygotic twins in our study were not concordant. Only the elder brother developed pure red aplastic anemia. Moreover, variant genes related to CVID susceptibility mainly inherited from their mother, but the mother only showed decreased bone density. It is suggested that these variants have an autosomal dominant inheritance with an incomplete penetrance. Li [29] carried out the WES on three sporadic CVID patients and their offspring and provided similar evidence. Some monogenic disease-causing mutations also appeared in their unaffected offspring.

In conclusion, we described the clinical phenotypes of monozygotic twins of CVID and tried to identify genetic defects using WES. It failed to identify a monogenic cause for CVID in the family, but we screened VDR, NHEJ1, DOCK5, NOD2 and C3 as candidate pathogenic genes. We speculated the potential pathogenic mechanism for combinatorial effects in CVID is inferred from their roles in T and B cell signal pathways activation (Shown in Fig. 2i). We supposed that the onset of twins may be caused by multiple

epistatic interactions [30] and cumulative effects, which means a polygenic pattern. Our study has some limitations. Firstly, the twins are unmarried and have no children for genetic testing. Secondly, the functional effects of these candidate mutants are not verified but have provided additional information for clinical management. Thirdly, it should be mentioned that other variants like non-coding variants or deep intronic variants resulting in splicing defects or epigenetics or CNV analysis may be the possibility because these variants cannot be detected in this WES study.

Since CVID forms a heterogeneous group of phenotypes and genotypes, genetic investigation is still a great challenge. A combination of clinical and genetic diagnosis can be more extensively utilized in the clinical practice of CVID. Disease-related gene variations need to be found and confirmed in more patient populations and further studies, which will help to profoundly improve our understanding of CVID.

Abbreviations

CVID: Common variable immunodeficiency; PRCA: Pure red cell aplastic; WES: Whole-exome sequencing; ICON: International Consensus Document; BMD: Bone mineral density; ALP: Alkaline phosphatases; PTH: Parathyroid hormone; SNPs: Single nucleotide polymorphisms; hg: Human genome; BWA: Burrows-Wheeler Aligner; PCR: Polymerase chain reaction; SIFT: Sorting Intolerant from Tolerant; CADD: Combined Annotation-Dependent Depletion; RT-qPCR: real-time quantitative PCR; SNVs: single nucleotide variants; MAF: minor allele frequency; VDR: Vitamin D receptor; NHEJ1: DNA non-homologous end-joining factor 1; DOCK5: dedicator of cytokinesis-5; NOD2: nucleotide-binding oligomerization domain 2; C3: complement 3; DBD: DNA-binding domain; VDREs: vitamin D-responsive elements; DNA-DSBs: DNA double-strand breaks; CRs: complement receptors; CTL: cytotoxic T lymphocyte.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

LS directed the study and contributed to planning of the treatment. XG analyzed and interpreted the patient's datasets, and was the major contributor in writing the manuscript. JH critically revised the article for important intellectual content. YW conducted bioinformatics analysis of data. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

All participants provided written informed consent and the study was approved by the Shanghai Jiaotong University School of Medicine Renji Hospital ethic review board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

a Family pedigree. Open symbols indicate healthy family members (I:3, I:4, II:1, II:3, II:4, II:5, II:6), whereas black filled symbols indicate individuals [III:1 (proband), III:2] diagnosed with CVID. Grey filled symbols indicate individuals diagnosed with malignancies. The cross indicates the deceased family members. The black arrow indicates proband. b Bone marrow smear of the proband with pure red aplastic anemia. The nucleated erythrocyte was only 1.5% (<5%) and early erythroblast was observable. c Filtering strategies for candidate SNVs in the study. The number of genes filtered was within the parentheses.

Figure 2

a VDR (Vitamin D receptor) exon2: c.71C>A mutation by Sanger sequencing. b VDR mRNA expression decrease in III:1, III:2 and II:4 who carry the mutation. c NHEJ1 (DNA non-homologous end-joining factor 1) mRNA expression decrease in III:1, III:2 and II:4 who carry the mutation. d Functional domains of VDR protein. e Mutated VDR protein secondary structure prediction by using HOPE online. The result suggested that the mutation may lead to greater hydrophobicity and may change the interaction between the protein and the corresponding protein. f Mutated NHEJ1 protein secondary by using HOPE online. g Tertiary structure prediction of mutated NHEJ1 protein. In the tertiary structure prediction, both the wild-type and the mutant residue are shown in green and red respectively. The rest amino acids are shown in grey. h NHEJ1 exon4: c.475A>G mutation by Sanger sequencing. i Speculated pathogenic mechanism. The picture shows the proteins encoded by the selected candidate genes involved in the development and

maturation of B cells, as well as the role and interaction of T cell and B cell signal transduction. During B cell development, pre-B cells undergo V(D)J rearrangement, which triggers RAG-induced DNA double-strand damage repair (RAG-DSBs). Similarly, activated B cells undergo class-switching reorganization. This process triggers AID-induced DNA Double-strand damage repair (AID-DSBs). In both of the above processes, NHEJ1 is a key protein involved in DNA double-strand damage repair. Abnormal activation of the nuclear factor- κ B (NF- κ B) has been reported to be related to the pathogenesis of COVID. VDR protein is a membrane protein that regulates calcium ions positively after being activated, which further activate NF- κ B and the nuclear factor of activated T cells transcription factor (NF-AT) signaling pathways. NOD2 protein also can regulate NF- κ B signaling pathway. DOCK5 can play an immunoregulatory role by stimulating the BCR signaling pathway. As for the complement C3 protein, its cleavage product C3b can be combined with the BCR costimulatory factor CD21 to activate the BCR signaling pathway, and ultimately promote NF- κ B signaling pathway to regulate the immune response.