ORIGINAL ARTICLE

Congenital dyserythropoiesis anemia type Ia with a novel CDAN1 mutation diagnosed by whole exome sequencing

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Abstract

Background: Congenital dyserythropoiesis anemia type Ia (OMIM:224120), is a rare hereditary anemia. The diagnosis is difficult to make and usually delayed in part due to its rarity and nonspecific clinical manifestations.

Methods: Whole exome sequencing was applied for the genetic diagnosis of a 12-year-old boy who has suffered from hemolytic anemia since birth and who requires regular transfusions. Sanger sequencing of the variants detected in whole exome sequencing was performed in the patient and his parents.

Results: Compound heterozygous mutations of CDAN1 gene, including one previously reported and one novel mutation, which is a splicing change, were detected in the whole exome sequencing and confirmed by Sanger sequencing. The autosomal recessive inheritance was confirmed by pedigree analysis.

Conclusion: To our knowledge, this is the first case report of congenital dyserythropoiesis anemia type Ia with genetic diagnosis to be located in Taiwan. Because of the rarity of CDA Ia and the overlapping of the clinical manifestations with other hereditary anemias, the next-generation sequencing approach is effective for conclusive diagnosis of CDA Ia.

KEYWORDS
congenital dyserythropoiesis anemia, hemolytic anemia, hyperbilirubinemia, whole exome sequencing

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INTRODUCTION

Congenital dyserythropoiesis anemia (CDA) is a group of rare hereditary hemolytic anemias characterized by ineffective erythropoiesis and morphological abnormalities of erythroid precursors in the bone marrow (Iolascon, Esposito, & Russo, 2012; Iolascon, Russo, & Delaunay, 2011). CDAs are classified into type I (CDA I), type II and type III, based on the dysplastic changes observed in bone marrow erythroblasts by means of light and electron microscopy, the pattern of inheritance, and the associated dysmorphisms (Fujino et al., 2013). Although CDAs have traditionally been defined in morphological terms, they have come to be genetically defined in recent years. CDA Ia is an autosomal recessive disease characterized by erythroid hyperplasia, binuclear erythroblasts, and chromatid bridges between the nuclei of erythroblasts in bone marrow light microscopic (LM) examinations and a spongy appearance of heterochromatin of erythroblasts in electron microscopic examinations (Iolascon, Heimpel, Wahlin, & Tamary, 2013). Patients with CDA Ia would present moderate to severe macrocytic anemia, jaundice, splenomegaly, and iron overload. Skeletal abnormalities of distal limbs have been described (Tamary et al., 2005). The anemia and jaundice were usually recognized in neonatal or early childhood situations (Heimpel et al., 2006; Shalev et al., 2004; Shalev, Tamary, Shaft, Reznitsky, & Zaizov, 1997). However, such diagnosis is difficult to make, and it is often delayed because of its rarity and obscure erythroblast abnormalities, in which case it would take an experienced expert to think of such a possibility or an occurrence. Here, we present the case of a 12-year-old boy with transfusion-dependent hemolytic anemia since infancy and genetically diagnosed as CDA Ia with compound heterozygous CDAN1 (CDAN1; 607465) mutations, one of which was previously reported and the other novel variant determined using whole exome sequencing.

MATERIALS AND METHODS

2.1 Ethical compliance

This study was approved by the Institutional Review Board-II Kaohsiung Medical University Chung-Ho Memorial Hospital (KMUHIRB-G(II)-20160043).

The 12-year-old male patient with clinically diagnosed with congenital hemolytic anemia was enrolled for whole exome sequencing (WES) analysis. Sanger sequencing for possible disease-causing variants were performed for the patient and his parents. The detailed methods of WES and Sanger sequencing were as previously described (Lin et al., 2018). The primer sequences for Sanger sequencing of the two CDAN1 variants were: exon14 forward: 5′-CGGTCTCTGGA TGTCGGGACT-3′; exon14 reverse: 5′-GCCAGCCCAAGGACAGCAAGT-3′; exon18 forward: 5′-GACAATGGCGGCT GTGTTGGA-3′; exon18 reverse: 5′-GCAGGCCCTGGCTGGTCT-3′. The GenBank reference sequence and version number for CDAN1 was NM_138477.4.

RESULTS

This 12-year-old male patient was diagnosed with hemolytic anemia since birth. He was a prematurity with low birth weight (gestational age: 36 + 2 weeks, birth body weight: 1,950 mg). Delayed crying, pale appearance, poor physical activity, and grunting were noted at birth. Severe anemia (Hemoglobin (Hb): 4 g/dl), hyperbilirubinemia (total bilirubin: 5.5 g/dl), and highly elevated lactate dehydrogenase (LDH: 5,908 U/L) were noted. Dyspnea with desaturation developed soon after birth and intubation with mechanical ventilation support were given. Echocardiogram showed a large patent ductus arteriosus with right heart failure. Abdominal ultrasound showed hepatomegaly. Blood exchange transfusion was performed for severe anemia and hyperbilirubinemia. Small left fourth toe and malposition of right fourth toe were also noted. Chromosome study of the peripheral blood revealed normal karyotype (46 XY). After intensive care, PDA reduced, right heart failure subsided, and anemia improved (Hb: 10.9 g/dl). He was discharged at the age of 1 month. At the age of 2 months, severe anemia (Hb: 3.9 g/dl) developed again and a blood transfusion was performed. Subsequent to the initial transfusion, regular visits to the hematology department were made. Blood transfusions were administered intermittently for 1 year; however, low Hb levels (around 5–6 g/dl) and poor body weight gain were noted. Bone marrow aspiration was performed and erythroid hyperplasia was impressed. Regular transfusion with targeted trough Hb levels above 10 g/dl started post 1-year-old. A splenectomy was performed at the age of 6 years in an attempt to reduce transfusion requirement under the impression of transfusion-dependent anemia, but the eventual requirement for transfusion was not reduced significantly.

Red blood cell (RBC) indices taken 2 weeks after latest blood transfusion showed normocytic anemia (RBC count: 2.66 × 10^6/ul; Hb: 8.0 g/dl; MCV: 88.7 fl; MCH:30.1 Pg; MCHC: 33.9 g/dl). The reticulocyte count was 2.2% (reference range: 0.87–2.26%) and the reticulocyte production index was 0.64. The peripheral blood smear showed macracytic RBC, shistocytes, bite cells, and target cells (see Figure 1). Direct and indirect anti-globulin test were both negative. Plain film of his left foot showed the absence of left third middle and distal phalanges.

WES using DNA extracted from the mononuclear cells of the peripheral blood was performed. The workflow of analysis...
of WES data and the variants of the coding regions in genes of RBC membrane disorders, hyperbilirubinemia, and other congenital hemolytic anemia discovered were annotated in Figure 2. For heterozygous variants in genetic diseases, the variant allele frequency in genomic DNA from tissue approaches 50% in general. To screen more variants, and to avoid the sequencing bios and alignment bios, we selected variants with a ratio no less than 30%. The 525 variants with a ratio no less than 30% and global minor allele frequency less than 1% were listed in supplement (Data S1). The panels of the target genes for searching the possible causative variants were published previously (Lin et al., 2018). A heterozygous CDAN1 variant (c.2140C>T, p.R714W) in exon14 was detected (sequence depth/ratio of variant: 29/59 (49.15%)). Sanger sequencing of the patient and the parents showed a paternal origin of this variant. Previous literature showed that CDAN1 mutations was associated with CDA I. All proven cases of CDA I reported were autosomal recessive, including homozygous or compound heterozygous mutations involving coding and/or splicing regions. Tamary et al. reported 12 polymorphic sites of the CDAN1 gene in their study and none of them were found in the WES result of our patient (Tamary et al., 2005). Therefore, we re-analyzed the WES data for searching for variants of CDAN1 splicing regions. A CDAN1 variant (NM_138477:exon18:c.2408-2A>G), which was predicted as interfering with splicing, and which has not been previously reported or listed in the Human Gene Mutation Database (HGMD), was detected (sequence depth/ratio of variant: 12/32 (37.50%)). The exon18:c.2408-2A>G likely affects the acceptor splice site of exon 18, which may result in exon18 skipping or the usage of the cryptic exonic or intronic splice sites that leads to the inclusion of an intron fragment or exon fragment skipping (see Figure 3; Anna & Monika, 2018). Sanger sequencing of the patient and the parents showed a maternal origin of this variant. Finally, the patient was diagnosed genetically as CDA Ia with compound heterozygous CDAN1 mutations (c.2140C>T, p.R714W and exon18:c.2408-2A>G; see Figure 4).

4 | DISCUSSION

CDA Ia was more prevalently reported in the population of Israeli Bedouin and of Europeans (Heimpel et al., 2006; Tamary et al., 1996). Single cases have been similarly reported in the United States, Japan, India, and China (Fujino et al., 2013; Kawabata et al., 2012; Kumar, Kushwaha, & Singh, 2014; Ru et al., 2014, 2008; Wang et al., 2018; Wickramasinghe & Wood, 2005). The estimated cumulative incidence of CDA Ia in Europe is approximately 0.24/million (Iolascon et al., 2012). To date, 63 CDAN1 mutations were recorded in The Human Gene Mutation Database, including 42 missense mutations, 4 splicing mutations, 16 small deletions/insertions, and 1 gross deletion. Although no particular frequent mutations were reported, the majority of patients showed mutations of at least one allele from exons 6 to 28 (Iolascon et al., 2012). No phenotype–genotype correlation was established. The disease diversities noted in the homozygous Bedouin patients and the existence of patients with phenotypic CDA Ia in which no mutations of CDAN1 gene have been found to suggest the presence of a promoter defect or modifier genes (Dgany et al., 2002; Iolascon et al., 2012). Our patient showed a compound heterozygous CDAN1 mutations (c.2140C>T, p.R714W and exon18:c.2408-2A>G). The missense mutation (c.2140C>T, p.R714W) has been reported in European descents and Chinese (Dgany et al., 2002;
Whole exome sequencing (WES) Variant Data
693288 variants identified

Ratio of variant >=30%
30324 variants identified

Global minor allele frequency <1%
525 variants identified

ClinVar Annotation

Pathogenic
1 variants identified

Benign
3 variants identified

Uncertain
521 variants identified

dbSNP Annotation
264 variants identified

Pathogenicity predictions
-SIFT, PolyPhen, CADD

Damaging/Tolerated
411 variants identified

HGVs format Annotation
520 variants identified

Variants selection*

1 variant identified

Sanger sequencing confirmation and pedigree analysis

*Variants of target genes, include genes of RBC membrane disorders, hyperbilirubinemia and other congenital hemolytic anemia, were selected.

FIGURE 2 The workflow of analysis of whole exome sequencing data and the variants in the coding regions identified in every step were annotated
FIGURE 3  The exon 18 intron/exon boundary sequences of wild type (A) and the exon18:c.2408-2A>G (B) were shown. The exon18:c.2408-2A>G likely affects the acceptor splice site of exon 18, which may result in exon18 skipping (a) or the usage of the cryptic intronic (b) or exonic (c) splice sites that leads to the inclusion of an intron fragment or exon fragment skipping. (3’-ss, 3’-splicing site; ex18 frag, exon 18 fragment; in, intron fragment; new ss, new splicing site). Adapted from figure 3, Anna and Monika (2018)

FIGURE 4  The electrophorograms indicate compound heterozygous CDNA1 mutations (c.2140C>T, p.R714W and exon18:c.2408-2A>G) of the patient and the origins of the mutations. The GenBank reference sequence and version number for CDAN1 was NM_138477.4
Tamary et al., 2005; Wang et al., 2018). Splicing mutants were found in 13% of patients with CDA Ia; and, as such, we found a novel splicing mutant present in our patient.

In the German CDA Registry, the age of diagnosis ranged from 0.1 to 45 years (median, 17.3 years). Prior incorrect diagnosis was noted in 30% of their 74 patients with CDA Ia, including pernicious anemia, iron-deficient anemia, thalassemia, or unclassified congenital hemolytic anemia (Heimpel et al., 2006). On the contrary, in an Italian study using NGS based multigene panel testing for patients with hereditary anemias, most of the patients with a nonmatched, clinical diagnosis, and genetic mutations were previously suspected to have suffered from CDA, and nearly half of them exhibited a conclusive diagnosis of enzymatic defects, such as pyruvate kinase deficiencies (Russo et al., 2018). Molecular studies searching the CDAN1 gene mutations are helpful for making a conclusive diagnosis (Heimpel et al., 2006; Iolascon et al., 2013).

Our patient showed a congenital hemolytic anemia with inadequate reticulocyte count, persistent indirect hyperbilirubinemia, elevated LDH, and feet deformities. The bone marrow smear showed an erythroid hyperplasia; however, the characteristic nuclear bridging, nuclear lobulations, and multinuclearity of erythroblasts were subtle enough to make the diagnosis difficult (see Figure 1). Although most of the patients with CDA Ia showed moderate hemoglobin levels, some transfusion-dependent patients have been reported (Ayas et al., 2002; Parez et al., 2000; Wickramasinghe et al., 1998). A splenectomy is considered to be in vain in order to increase the patient's hemoglobin levels. Long-term complications include iron overload, liver cirrhosis, heart failure, endocrine dysfunction, extramedullary bulk, and leg ulcers (Heimpel et al., 2006). Complicated pregnancies have been reported (Bank, Ermens, Van Der Linden, & Brand, 2012; Shalev et al., 2008). Hematopoietic stem cell transplantation (HSCT) or interferon-α have been successfully applied in transfusion-dependent CDA Ia cases (Ayas et al., 2002; Heimpel et al., 2006; Iolascon et al., 2013). Our patient did not receive HSCT or interferon-α treatment due to the lack of precise diagnosis of a disease before this WES approach study. Correct diagnosis for patients with CDA Ia is important for optimal management purposes. Our group has applied the WES approach with targeted gene panel analysis in the genetic diagnosis of red cell membrane disorders (Lin et al., 2018). In this particular patient, we applied a similar approach and successfully discovered the causative gene mutations as a result. Due to the rarity of CDA Ia, and the overlapping of the clinical manifestations with other hereditary anemias, a next-generation sequencing approach is effective to afford a conclusive diagnosis of CDA Ia.

In conclusion, we present the first case report of CDA Ia in Taiwan. Molecular study of patients with hereditary hemolytic anemia significantly assists in definite diagnosis, disease management and genetic counseling.

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CONFLICT OF INTEREST
The other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS
JGC and PCL conceived of the study and participated in its design and coordination. Clinical data acquisition and analysis were carried out by CNC. YHT, YSC, and CYL carried out the laboratory experiments. HYH conducted the bioinformatics analysis. PCL and CNC wrote this manuscript. All authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT
Authors elect to not share data due to privacy/ethical restrictions. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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