CASE REPORT

New Codanin-1 Gene Mutations in a Italian Patient with Congenital Dyserythropoietic Anemia Type I and Heterozygous Beta-Thalassemia

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Received: 1 April 2015 / Accepted: 16 December 2015 / Published online: 5 January 2016
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Abstract Congenital dyserythropoietic anemia type I is an autosomal recessive disorder associated with macrocytic anemia, ineffective erythropoiesis, iron overloading and characterized by abnormal chromatin ultrastructure in erythroblasts such as internuclear chromatin bridges, spongy heterochromatin and invagination of the nuclear membrane. A 58-year-old Caucasian man with chronic hemolytic anemia, heterozygous for β^+ -globin IVS1, nt110 G>A mutation (causing abnormal alpha:beta globin chain ratio) showed clinical, laboratory and hematological features suggesting diagnosis of CDA1. Sequence analysis of CDA-related genes revealed compound heterozygosity for two novel mutations in the CDAN1 gene: a frameshift mutation 3367 del 4 (TTAG) in exon 25 and a missense mutation c.1811 G>T in exon 11 causing an aminoacid change from glycine to valine at codon 565 (G565V). One of the propositus' brothers showed the same gene mutations. As the CDA1 can mimic thalassemia, a frequent misdiagnosis is possible especially in countries where the prevalence of thalassemia is high. A strong clinical suspicion in patients who do not reveal a clear genetic basis for presumed thalassemia may help clinch the correct diagnosis.

Keywords Congenital dyserythropoietic anemia · Ineffective erythropoiesis · CDAN1 gene · Internuclear chromatin bridges

Introduction

CDA1 belongs to a group of rare hereditary disorders characterized by ineffective erythropoiesis as predominant mechanism of anemia and by distinct morphological abnormalities of the majority of erythroblasts in the bone marrow.

The most common clinical manifestations are congenital anemia, jaundice, splenomegaly, and reduced reticulocyte production in contrast to the erythroid hyperplasia in the bone marrow. These findings may explain the difficulties in diagnosing this syndrome and distinguishing it from other congenital anemias like CDA type II and III, thalassemia and hemoglobinopathies. According to their morphological appearances, three forms of CDAs have been originally described (types I to III). The responsible genes have been identified as *CDAN1* [1], *SEC23B* [2] and *KIF23* [3], respectively.

CDA1 is an autosomal recessive disorder characterized by a moderate-to-severe macrocytic anemia. Bone marrow aspirates show binuclear intermediate and late-erythroid precursors as well as internuclear chromatin bridges. Ultrastructural erythroid features include spongy heterochromatin and invagination of the nuclear membrane, carrying cytoplasm and cytoplasmic organelles into the nucleus. Arrest of DNA synthesis [4] and apoptotic features in erythroid precursors have been described [5]. More than 30 unique mutations have been identified so far [6] in CDA1 patients. Approximately, 90 % of the patients with a bone marrow suggesting CDA type 1 have mutations in *CDAN1* [1]. Most patients with a confirmed diagnosis of CDA type 1 show mutations of at least one allele within *CDAN1*. The majority of mutations in *CDAN1* are missense or nonsense, and only two frameshift mutations are known [6]. We describe a case of CDA type 1, previously

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misdiagnosed as thalassemia intermedia, in which we identified two novel mutations in *CDAN1* gene.

Case Report

An Italian man 58 years old, born in Sicily, was admitted to our Hospital with a history of haemolytic anaemia. He was diagnosed, in another Hospital, with thalassemia intermedia at the age of 2 because of hypochromic microcytic anaemia with Hb levels between 7 and 9 gr/dl and with a very low transfusional requirement of 72.8 ml/kg/year.

Over the last year, his blood requirement was 129.6 ml/kg/year, maintaining a pre-transfusional Hb level of 9.0 g/dl.

Main clinical complications due to secondary hemochromatosis were insulin-dependent mellitus diabetes, hypothyroidism, severe osteoporosis and restrictive cardiomyopathy. Previous episodes of right ventricular failure and of atrial fibrillation were reported.

The patient had been treated with deferoxamine since 1972. Recently, his chelation treatment was changed to a combination of deferiprone 83 mg/kg and deferoxamine 50 mg/kg.

The patient was splenectomized at the age of 11 years. Meningitis was diagnosed and treated at the age of 17 years. Cholecystectomy was performed at the age of 37 years.

One of the propositus' brothers (II-4) (Fig. 1) too had had severe transfusion dependent anemia (Hb 6.5 gr/dl) since the age of 45. He died of sepsis and severe liver failure with portal hypertension. This brother was splenectomized in December 2000. His DNA was extracted for the molecular analysis and stored in our lab. The other members of the family did not show any clinical findings (Fig. 1).

The MCV was 85,1 fl; reticulocyte count, corrected for hematocrit, was 2.2 %; RDW was 14,4 % and blood smear showed anisopoikilocytosis with basophilic stippling.

Haptoglobin was 46.5 mg/dl (normal range 36–195 mg/dl); bilirubin total was 2.2 mg/dl; Lactate dehydrogenase and ferritin were increased to 806 U/l and 1118.9 ng/ml, respectively. Flow cytometric eosin-5-maleimide dye binding test revealed normal mean channel fluorescence (MCF) of red cells as 63.3 (normal range of our laboratory is 48.3–63.7) [7].

Further diagnostic testing of the index case revealed heterozygous β^+ -globin IVS1, nt110 G>A. Triple alpha genes, spherocytosis, hemolytic autoimmune anemia and deficiency of red cells enzymes were ruled out by laboratory tests. In 2013, during a hospitalization due to a ventilatory impairment syndrome, a bone marrow aspiration procedure was performed. CDAI was suspected when it showed megaloblastoid erythroid hyperplasia as well as some binucleated red cell precursors and inter-chromatin bridges (Fig. 2). The *CDAN1* gene was analyzed to confirm diagnosis. The genetic analysis of *CDAN1* gene was extended to all members of the family (Fig. 1).

Genomic DNA was extracted from mononuclear cells of peripheral blood samples by phenol–chloroform method. Coding sequences and the exon/intron boundaries of the *CDAN1* gene were amplified using the Taq polymerase system (Invitrogen-2,5U/50 μ l-reaction). Cycling conditions were as follows: 94 °C at 5 min, 14 cycles of 20 s at 94 °C, 40 s at 57 °C, 45 s at 72 °C; and then 25 cycles of 25 s at 94 °C, 40 s at 50 °C, 45 s at 72 °C and a final 7 min at 72 °C.

Polymerase chain reaction (PCR) products were sequenced directly using Big-Dye terminator 3.1 cycle sequencing kit and run on ABI PRISM 3130 DNA analyzer. Primers used for PCR and sequencing were designed in our laboratory (Table 1). The mutational analysis revealed two novel mutations in our patient: a frameshift mutation 3367 del 4 (TTAG) in exon 25 and a missense mutation Gly 565 Val in exon 11 (Fig. 3). The complete

Fig. 1 Family pedigree of the patient. **I** 1–2 obligate carriers (dead). **II** 1 healthy male (3367Del4(TTAG)/N); 2 proband (3367Del4(TTAG)/G565V); 3 healthy male (G565V/N); 4 affected male (dead) (3367Del4(TTAG)/G565V); 5 healthy female (G565V/N)

Fig. 2 Light microscopy of bone marrow aspirate. Erythroblasts indicating the spectrum of nuclear abnormalities (a) and internuclear chromatin bridging in CDAI (b) stained with May Grunwald-Giemsa 1000× original magnification

Table 1 Primers sequences used for amplification and sequencing are shown

	Primer sequences (5' > 3')
CDAN 1 ex 11 forward	TTTGTTTCAGTCTTCCTCCC
CDAN 1 ex 11 reverse	AGGCAAAGGAGACTGGAGAAC
CDAN 1 ex 25 forward	AGACCTGATAACCA AGCCAC
CDAN 1 ex 25 reverse	TTGCTTGTACCCAACCCTGAG

coding sequence was sequenced to exclude any other mutations. The propositus' deceased brother showed the same gene mutations (II-4, Fig. 1). Whereas, other members of the family were heterozygotes for *CDAN1* mutations (Fig. 1). Especially one of the two brothers and the sister were heterozygous for the mutation C1811 C>T and the other brother showed the mutation of 3367 del 4 (TTAG). None of them were healthy carrier of thalassemia while the deceased brother was heterozygous for β^+ -globin IVS1, nt110 G>A mutations.

One-hundred normal individuals (200 alleles) were subsequently analyzed using the same sequencing method described above, to verify if the genetic variants identified in the patient were mutations or simply polymorphisms. None of them showed the two DNA variants detected on the *CDAN1* gene of this family.

Discussion

CDAI is a rare clinical hereditary disease reported mainly from the Central and Western European countries and North Africa [8]. Very few cases have been reported from Indian Subcontinent [8]. The correct diagnosis is often delayed to adolescence or adulthood, when significant iron overload and end organ damage may have been occurred.

Patients have been misdiagnosed with hereditary spherocytosis (HS), myelodysplasia, thalassaemia intermedia, hereditary hemochromatosis or an unclassified congenital haemolytic anaemia [9]. Inaccurate diagnosis can lead to inappropriate therapies, such as iron supplements, aggressive transfusions or splenectomy. Fortunately the identification of the mutated genes involved in the majority of CDA patients in recent years has improved the possibility of detecting these diseases.

The exact cellular role of codanin-1, encoded by *CDAN1* gene is still unknown. Renella et al. [10] suggest that *CDAN1* mutations could lead to abnormal deposition on chromatin and subsequently cause a secondary inappropriate accumulation of Heterochromatin Protein 1 alfa (HP1alfa) in the Golgi apparatus of erythroblasts. Moreover, other authors [11] characterize Codanin-1 as an interaction partner of the histone chaperone anti-silencing function 1 (Asf-1) acting as a negative regulator of S-phase histone supply.

It remains unclear why erythropoiesis is particularly sensitive to Codanin-1 mutations, since Codanin-1 is an ubiquitously expressed protein [1, 10]. The majority of mutations are missense and nonsense and only two frameshift mutations are known. As previously noted, [1] no homozygous patients for null-type mutations were identified, suggesting that the absence of codanin-1 protein is lethal for the fetus.

Unlike thalassemia, no phenotype–genotype correlations can be made to date [12]. CDAI may show moderate to severe phenotypes. However, the clinical expression, according to some authors, could be due to factors determining “normal” variability of erythropoiesis rather than to the codanin-1 mutations [11]. In our case report, the propositus was even heterozygote for β^+ -globin IVS1, nt110 G>A mutation. To the authors knowledge in the

Fig. 3 Detail of *CDAN1* gene sequencing: **a** Frameshift mutation 3367 del 4 (TTAG) in exon 25 and **b** substitution in exon 11 (GGC>GTC) resulting in amino acid change G565V

recent literature similar cases have never been described, and we hypothesize that our patient could have a more severe clinical phenotype due to beta thalassemia mutation. However, it is difficult to show how heterozygosity for β^+ -globin IVS1, nt110 G>A mutation may influence erythropoiesis in this case of CDA1.

In conclusion, the delayed diagnosis in our patient suggests that the hematologist must be careful whenever a patient is suspected of having thalassemia intermedia without a clear genetic basis. In such cases, if the clinical picture, the hematological tests and the light microscopy of bone marrow are suggestive of one of the classical I to III CDA types, appropriate genetic tests should be performed for confirmation.

Our report expands the spectrum of genetic lesions reported in *CDAN1* gene in literature, and is especially important as polymorphisms have definitely been excluded by sequencing studies on normal individuals.

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