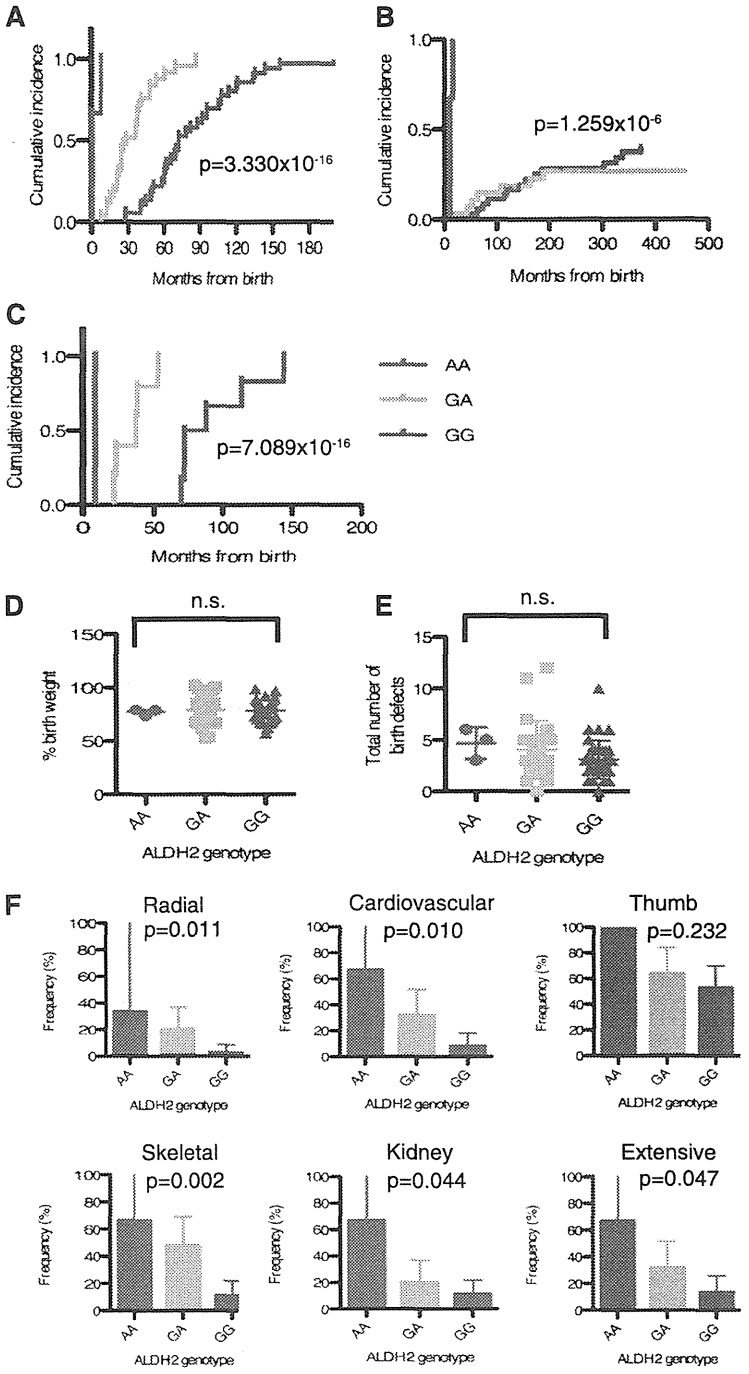

Figure 1. Effects of the ALDH2 deficiency on Japanese FA patients. (A-B) Cumulative incidence of BMF (A) or MDS/AML (B) were analyzed in 64 FA subjects. Numbers of AA, GA, and GG patients were 3, 25, and 36, respectively. (C) Cumulative incidence of BMF was analyzed in patients with confirmed biallelic *FANCA* mutations having protein truncations and/or large deletions (n = 12). Numbers of AA, GA, and GG patients were 1, 5, and 6, respectively. *P* values shown were calculated by the Gray test. In panel A, *P* values between genotypes were 8.625×10^{-7} (GG vs GA), 2.107×10^{-10} (GG vs AA), 1.259×10^{-6} (GA vs AA), respectively. In (B), the difference between GG and GA subjects was not significant ($P = .4564479$), whereas other statistical comparisons were highly significant (GG vs AA, 2.911×10^{-10}; GA vs AA, 8.813×10^{-8}). In panel C, the *P* values between GG and GA, GG and AA, or GA and AA were calculated as 0.001228433, 0.01430588, 0.02534732, respectively. (D) Percentage of birth weight or (E) total number of physical abnormalities (shown in supplemental Table 1) in 64 FA patients with 3 *ALDH2* genotypes. Birth weight was normalized to mean weight at gestational age in Japan. Mean and SEM are indicated. Birth weight records were missing for 3 patients (supplemental Table 1). There was no significant difference between the *ALDH2* genotypes (Kruskal-Wallis test). (F) Frequency (percentage) of cardiovascular, radial, thumb, skeletal, kidney, and extensive malformations in each *ALDH2* genotype. *P* values were calculated by the Cochran-Armitage test for trend, which detects statistical significance of effects across the genotypes. The error bars represent 95% confidence intervals.

Acknowledgments

The authors thank the individual patients and families in the study who made this work possible, Dr K. J. Patel (University of Cambridge) for communicating unpublished results, Dr James Hejna (Graduate School of Biostudies, Kyoto University) for critical reading of the manuscript and English editing, Mr Naoya Suzuki and Drs Akira Niwa and Megumu Saito (CiRA, Kyoto University) for discussion, and Ms Fumiko Tsuchida, Emi Uchida, Sumiyo Ariga, Chinatsu Ohki, and Mao Hisano for expert technical assistance.

This work was supported by grants from the Ministry of Health, Labor and Welfare, and grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Authorship

Contribution: M.Y. and H.Y. examined DEB-induced chromosome aberrations, carried out MLPA testing, and analyzed clinical records; K.Y., Y.O., Y.S., K.C., H.T., S.M., S.K., and S.O.

performed WES and analyzed sequence data; A.H. validated exome data and carried out genotyping; A.H., M.Y., H.Y., K.M., J.N., and M.T. analyzed data; and M.Y., M.T., and K.M. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Int J Hematol (2013) 97:650–653
DOI 10.1007/s12185-013-1338-4

CASE REPORT

Congenital dyserythropoietic anemia type 1 with a novel mutation in the CDAN1 gene previously diagnosed as congenital hemolytic anemia

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Received: 16 January 2013 / Revised: 9 April 2013 / Accepted: 11 April 2013 / Published online: 19 April 2013

Abstract The congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of genetic disorders of red cell production. They are characterized by ineffective erythropoiesis and dyserythropoiesis. Here, we present the clinical description and mutation analysis of a Japanese female with CDA type 1. She has long been diagnosed with unclassified congenital hemolytic anemia from the neonatal period. However, bone marrow morphology and genetic testing of the CDAN1 gene at the age of 12 years confirmed the afore-mentioned diagnosis. Thus, we should be aware of the possibility of CDA if the etiology of congenital anemia or jaundice cannot be clearly elucidated.

Keywords Congenital dyserythropoietic anemia · CDAN1 gene · Congenital hemolytic anemia

Introduction

The congenital dyserythropoietic anemias (CDAs) comprise a group of very rare hereditary disorders characterized by ineffective erythropoiesis and distinct morphological abnormalities of the erythroblasts in the bone marrow [1]. Morphological analysis is the first step in the diagnosis of all types of CDA, followed by confirmatory tests [2]. The diagnosis of CDAs can be delayed due to their rarity and lack of information (especially in non-severe cases) [3–5].

On the basis of the dysplastic changes observed in bone marrow erythroblasts by light and electron microscopy, the mode of inheritance and the associated dysmorphism, CDAs have been divided into 3 major types: CDA types 1, 2, and 3. Responsible genes have been identified for CDA type 1 (*CDAN1*) [6] and CDA type 2 (*SEC23B*) [7], not for CDA type 3.

In this brief report, we describe a unique case of CDA type 1 previously diagnosed as unclassified congenital hemolytic anemia. Marked erythroid dysplasia and the detection of a novel mutation in the *CDAN1* gene aided in accurately diagnosing the condition.

Case report

A 12-year-old female was referred to our hospital for further evaluation of persistent anemia after gastroenteritis. She had no family history of hemolytic anemia, was born at 39 weeks' gestation, and weighed 2,085 g at birth. Her initial symptom was severe jaundice at birth. She received three exchange transfusions during infancy, followed by erythropoietin administration for subsequent anemia up to the age of 1 year. At the age of 8 years, she experienced exacerbations of anemia, jaundice, and splenomegaly following mild gastroenteritis. Evaluation of her laboratory results at that point revealed low hemoglobin levels (10.6 g/dl), elevated mean corpuscular volume (MCV 101.3 fl), elevated bilirubin levels (total bilirubin 3.1 mg/dl, direct bilirubin 0.9 mg/dl), and undetectable haptoglobin (<10 mg/dl). The clinical and hematological features were suggestive of congenital hemolytic anemia; however, further investigation

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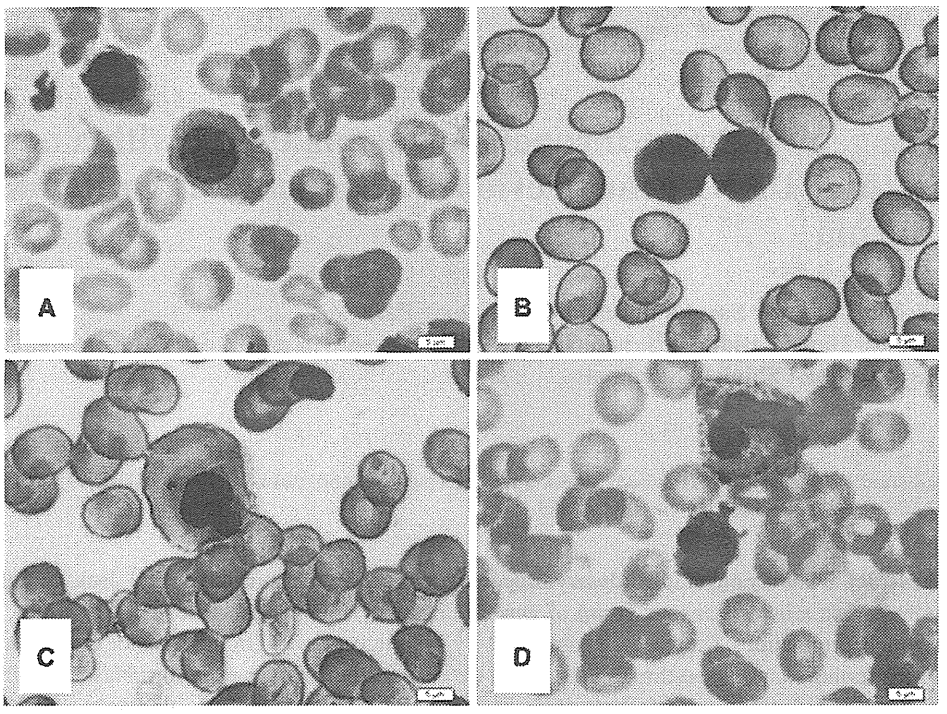

Fig. 1 Bone marrow morphology. **a** Megaloblastic changes, **b** nuclear bridging, **c** nuclear lobulations, and **d** multinuclearity (May–Giemsa staining, ×400)

[peripheral blood smear, osmotic fragility test, fraction of hemoglobin, isopropanol test, and red blood cell (RBC) enzyme activities] excluded the possibility of disorders of red cell membrane, thalassemias, unstable hemoglobinopathies, and red cell enzymopathies.

At the time of her first visit to our hospital, physical examination revealed mild splenomegaly and conjunctival pallor; she had no skeletal malformations (including distal limb anomalies). Laboratory evaluation revealed low hemoglobin levels (8.1 g/dl), normal MCV values (93.9 fl), normal bilirubin levels (total bilirubin 1.0 mg/dl, direct bilirubin 0.2 mg/dl), and mildly elevated serum ferritin levels (400.8 ng/ml). The levels of serum vitamin B12, folate, and iron were within the normal ranges. Furthermore, peripheral blood smear revealed anisocytosis and poikilocytosis (including teardrop-shaped poikilocytes), and schistocytes. Bone marrow examination revealed erythroid hyperplasia and marked erythroid dysplasia; megaloblastic changes, nuclear bridging, nuclear lobulations, multinuclearity were observed (Fig. 1). No significant features of dysplasia were observed in the myeloid or megakaryocytic lineages. To confirm the diagnosis, we conducted a mutational analysis that revealed a novel heterozygous frameshift mutation c.552_553 insG in exon 2, and another known [6] heterozygous missense mutation c.A1910G in exon 12 of *CDAN1* gene (Fig. 2); subsequently, we diagnosed her as a case of CDA type 1. One year after the diagnosis, her anemia resolved spontaneously (hemoglobin levels

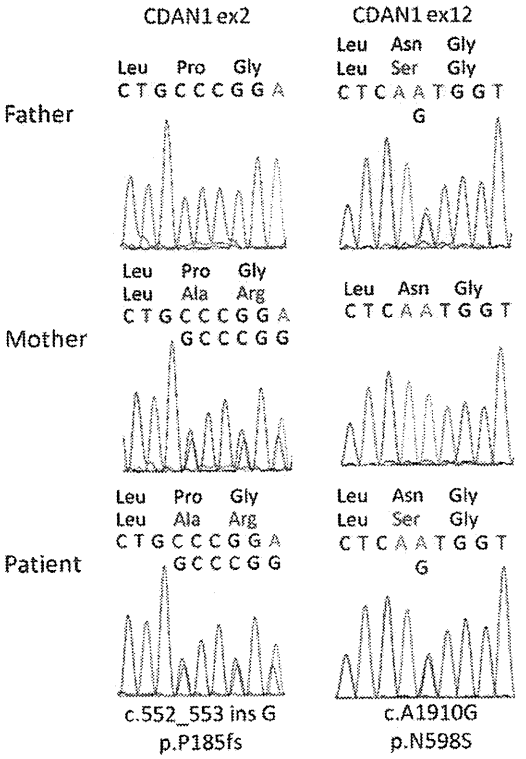

Fig. 2 The compound heterozygous mutation of the *CDAN1* gene

11.1 g/dl), but the ferritin levels remained relatively high (342.1 mg/ml); this required meticulous observation and follow-up.

Discussion

We report a 12-year-old female diagnosed with unclassified congenital hemolytic anemia with recurrent episodes of anemia and jaundice; subsequently, she was diagnosed with CDA type 1. CDA type 1 is inherited as an autosomal recessive disease. More than 150 patients have been described, mainly patients from Western Europe, the Middle East, India and Japan [8]. The anemia observed in CDA type 1 varies from mild to severe. About 50 % of neonates with CDA type 1 need at least one transfusion of erythrocytes, and some remain transfusion-dependent in the following years [9]. In most adolescents and adults, the need for transfusions is limited to aplastic crisis, pregnancy, periods of severe infections, or major surgery [10]. The anemia seen in CDA type 1 is usually macrocytic; in addition, peripheral blood smear showed other features of anisocytosis, poikilocytosis, and basophilic stippling [2]. Moreover, light microscopy of the bone marrow in CDA type 1 presents erythroid hyperplasia with abnormal precursors displaying a megaloblastoid appearance. Dysplastic signs include markedly irregular nuclei with frequent binucleate erythroblasts [11]. A particular diagnostic feature in CDA type 1 is thin, internuclear chromatin bridges between nearly completely separated erythroblasts.

Nevertheless, CDA should be diagnosed only after exclusion of other congenital anemias known to be associated with ineffective erythropoiesis and dyserythropoiesis [12]. Distinguishing CDA and the other congenital hemolytic anemias only on the basis of clinical course, laboratory data, and peripheral blood smear can be challenging. In CDA and the other congenital hemolytic anemia, symptoms of anemia and jaundice vary from mild to severe, with the most severe cases presenting in the neonatal period and milder cases presenting in adolescence or later stages in life. Abnormally shaped RBCs can appear in both the categories. Heimpel et al. [13] reported that in the German CDA Registry, the age of the 21 patients at the time of initial diagnosis of CDA type 1 ranged 0.1–45 years (median 17.3 years) and that 11 of 21 cases were previously misdiagnosed as congenital hemolytic anemia. Bone marrow examination might be often omitted, not usually performed, in pediatric cases with hemolytic anemia. In contrast, bone marrow examination is indispensible in case of CDAs because CDAs are diagnosed only after identifying distinct morphological abnormalities of the erythroblasts in the bone marrow.

Approximately, 90 % of patients with bone marrow evaluation suggesting CDA type 1 have mutations in *CDAN1* [6]. Most patients with a confirmed diagnosis of CDA type 1 demonstrate mutations of at least one allele from exons 6 to 28 within *CDAN1*; more than 30 unique mutations have been identified so far [6, 10, 13–17]. The majority of mutations in *CDAN1* are missense or nonsense, and only two frameshift mutations are known [10]. To our knowledge, c.552_553 insG in exon 2 is a novel frameshift mutation in *CDAN1*.

In summary, we report a Japanese female of CDA type 1. Bone marrow morphology and genetic testing in *CDAN1* gene was the key to accurate diagnosis. Taken together, when we encounter a patient whose clinical manifestations and laboratory results suggest the possibility of congenital hemolytic anemia but we cannot confirm the diagnosis, we should consider the possibility of CDA and bone marrow morphology and genetic testing should be conducted.

Conflict of interest The authors declare that they have no conflict of interest.

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