Gene 640 (2018) 73–78

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Identification of *CDAN1*, *C15ORF41* and *SEC23B* mutations in Chinese patients affected by congenital dyserythropoietic anemia

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ARTICLE INFO

Keywords:
Novel mutations
CDAN1
C15ORF41
SEC23B
Chinese CDA patients

ABSTRACT

Congenital dyserythropoietic anaemias (CDAs) are a group of rare haematological disorders characterized by ineffective erythropoiesis and dyserythropoiesis and reduced numbers of red cells, often with an abnormal morphology. Pathogenic defects in *CDAN1*, *C15ORF41*, *SEC23B*, *KIF23*, *KLF1* and *GATA1* genes have been identified in CDAs patients. In this study, we described 13 unrelated Chinese CDAs patients and identified 21 mutations, including 5 novel mutations in *CDAN1* gene, and 5 novel mutations in *SEC23B* gene. Additionally, we predicted the molecular consequence of these missense mutations with Polymorphism Phenotyping v2 (Polyphen), Sorting Intolerant From Tolerant (SIFT), MutPred (http://mutpred1.mutdb.org/) and Protein Variation Effect Analyzer (Provean, http://provean.jcvi.org/seq_submit.php) and analyzed the conservation of the mutated amino acid among proteins from several mammalian species.

1. Introduction

Congenital dyserythropoietic anemias (CDAs) are a group of rare heterogeneous genetic hematological disorders characterized by ineffective erythropoiesis. Three major types of CDAs, namely CDA I, CDA II and CDA III, have been identified based on the clinical manifestations and nucleotide variations (Wickramasinghe and Wood, 2005; Iolascon et al., 2013).

CDA I is an autosomal recessive disease which is caused predominantly by mutations in the *CDAN1* gene (Dgany et al., 2002). The major clinical manifestations of CDA I are jaundice, mild to transfusion-dependent macrocytic anemia, and high levels of serum ferritin caused by increased iron absorption secondary to erythroid hyperplasia and ineffective erythropoiesis (Wickramasinghe and Wood, 2005). Hallmarks of this disease include internuclear chromatin bridges and heterochromatin that has a spongy appearance. These features can be observed in bone marrow aspirates by light and electron microscopy respectively (Ru et al., 2014; Wickramasinghe and Wood, 2005). *CDAN1*, which encodes codanin-1, is involved in cell division. Codanin-1 interacts with the cytosolic Asf1-H3-H4-importin-4 complex, which is involved in nucleosome assembly and disassembly (Gambale et al., 2016). Thus defects in CDAN1 may impair DNA synthesis and chromatin assembly. Interestingly, 20% of CDA I patients do not have pathogenic mutations in their *CDAN1* gene. For example, deleterious homozygous missense mutations (p.Y94C and p.L178Q) in the *C15ORF41* gene have been identified in Middle-Eastern and South-East Asian origin families affected by CDA I (Babbs et al., 2013). *C15ORF41* encodes a novel endonuclease with two helix-turn-helix domains,

Abbreviations: CDA, congenital dyserythropoietic anaemias; PolyPhen, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant From Tolerant; MAF, minor allele frequency; Provean, Protein Variation Effect Analyzer; ExAC, Exome Aggregation Consortium; SNP, single nucleotide polymorphism; M, male; F, female; Ref, reference range; HGB, hemoglobin; MCV, mean corpuscular volume; RET, reticulocyte percentage; RBC, red blood cells; SF, serum ferritin; AA, amino acid; NA, not available; wt, wild-type; ins, insertion; del, deletion; dup, duplication

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http://dx.doi.org/10.1016/j.gene.2017.10.027
Received 7 June 2017; Received in revised form 18 September 2017; Accepted 10 October 2017
Available online 12 October 2017
0378-1119/

Table 1
Clinical features and pathogenic mutations in Chinese CDA patients.

ID	Gender	Onset years	HGB, g/L	MCV, fL	RET,%	RET, 10^{12}/L	RBC, 10^{12}/L	SF, ng/mL	Gene	Mutations		
										Exon	Nucleotide change	AA change
									CDAN1			
1	M	5	80	87.2	1.91	0.0508	2.66	542		14	c.2140 C > T	R714W/wt
										26	c.3389 C > T	P1130L/wt
2	M	25	52	81.4	5.04	0.1058	2.10	684		17	c.2393 G > T	C798F/wt
										19	c.2641 A > T	I881F/wt
										20	c.2671 C > T	R891C/wt
										23	c.3005–3008 dup CTGA	E1003fs/wt
2a	M	48	130	95	1.4	0.0619	4.42	120		23	c.3005–3008 dup CTGA	E1003fs/wt
3	M	37	72	115	1.87	0.0370	1.98	675		26	c.3389 C > T	P1130L/P1130L
4	M	0	56	110	2.71	0.0393	1.45	494		14	c.2044 C > T	R682X/wt
										24	c.3193 C > T	R1065W/wt
5	M	48	85	109	2.1	0.0508	2.42	NA		2	c.414–415 del GG	V140 fs/wt
										14	c.2029 C > T	R677W/wt
									C15ORF41			
6	F	37	48	117.1	4.23	0.0520	1.23	672		4	c.217 C > G	L73 V/L73 V
									SEC23B			
7	F	29	108	102.7	2.29	0.0774	3.38	380		1	c.74 C > A	P25H/wt
8	F	32	70	98.1	1.59	0.0343	2.16	NA		12	c.1467 C > G	H489Q/wt
										14	c.1727 T > C	F576S/wt
9	F	9	89	90.1	1.52	0.0462	3.04	NA		12	c.1467 C > G	H489Q/H489Q
10	M	1	70	84.1	1.17	0.0288	2.46	NA		12	c.1467 C > G	H489Q/wt
										16	c.1949 T > C	L650S/L650S
11	M	0	89	83.2	0.72	0.0222	3.09	NA		17	c.2129 C > T	T710 M/wt
										19	c.2254–2255 ins A	V753 fs/wt
12	M	0	81	108	1.9	0.0428	2.25	482		4	c.494 G > A	G165D/wt
										11	c.1352 G > T	C451F/wt
13	M	13	54	91.7	1.69	0.0286	1.69	342		12	c.1445 A > G	Q482R/wt
										12	c.1467 C > G	H489Q/wt
										17	c.2108 C > T	P703L/wt
13a	M	41	153	85.6	0.81	0.0423	5.22	32		NA	NA	NA
13b	F	40	136	93.7	0.71	0.0313	4.61	54		NA	NA	NA

Onset, the onset of symptoms was identified as the age when an individual acquires, develops or first experiences CDA-related symptoms; M, male; F, female; Data, clinical results of the patients; Ref, reference range; HGB, hemoglobin (Ref, M, 120–160 g/L; F, 110–150 g/L; Child, 120–140 g/L); MCV, mean corpuscular volume (adult, 80–95 fL; Child, 80–100 fL); RET, reticulocyte percentage (Ref, 0.5–1.5%); RET, absolute number of reticulocytes (Ref, 0.024–0.084 10^{12}/L); RBC, red blood cells (Ref, M, 4–5.5 10^{12}/L; F, 3.5–5 10^{12}/L; child, 4.0–4.5 10^{12}/L); SF, serum ferritin (Ref, 21.8–274.7 ng/mL); AA, amino acid; NA, not available; wt, wild-type; ins, insertion; del, deletion; dup, duplication.

which facilitate its interaction with DNA, and a nuclease domain (Babbs et al., 2013). Like codanin-1, it may also interact with Asf1b to influence nucleosome assembly and disassembly (Gambale et al., 2016).

CDA II is also an autosomal recessive disease and is the most common type of CDA. More than 450 cases have been reported from southern Italy, northwest Europe, north Africa and India (Iolascon et al., 2013; Russo et al., 2014; Wickramasinghe and Wood, 2005). CDA II is caused by mutations in the *SEC23B* gene, which encodes the secretory COP II component of SEC23B (Bianchi et al., 2009; Schwarz et al., 2009). The main clinical symptoms of CDA II are normocytic anemia of varying severity (which may even be transfusion dependent), jaundice and hepatosplenomegaly. Bone marrow examination reveals 10–35% binucleated late erythroblasts due to erythroid hyperplasia, and red cells with a double plasma membrane, both important microscopic features of this disease (Heimpel et al., 2010; Wickramasinghe and Wood, 2005). Furthermore, ineffective erythropoiesis may also lead to secondary iron overload in CDA II patients (Liu et al., 2012; Russo et al., 2016). SEC23B is a core component of coat protein complex II, which is responsible for vesicular transport from the endoplasmic reticulum (ER) to Golgi apparatus (Khoriaty et al., 2012). SEC23B may also be involved in telophase during cell division as a component of the midbody (Gambale et al., 2016). Therefore, the typical CDA II feature of bi- and multi-nucleated erythroblasts may result from the impairment of cell division.

Apart from these two major types of CDA, around fifty cases of CDA III and other CDA subgroups, such as CDA IV and variants, have been reported. CDA III and IV are inherited in an autosomal dominant manner and the pathogenic genes underlying these diseases are *KIF23* (Liljeholm et al., 2013) and *KLF1* (Iolascon et al., 2012), respectively. However, autosomal recessive or X-linked inheritance patterns have also been documented in some CDA variants, and mutations in *GATA1* or other genes may be responsible for these diseases (Iolascon et al., 2013; Iolascon et al., 2012).

A wide spectrum of mutations has been identified, mainly in European countries, and, to date, only around twenty Chinese CDAs cases were reported (Ru et al., 2014). In the current work, we studied 13 unrelated Chinese CDA patients and reported 21 mutations (10 novel mutations) in the *CDAN1*, *C15ORF41* and *SEC23B* genes. In addition, we carried out the prediction of the molecular consequence of these missense mutations identified in our work with Polyphen (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), MutPred (http://mutpred.mutdb.org/) and Provean (http://provean.jcvi.org/seq_submit.php) and analyzed the conservation of the mutated amino acid among proteins from several mammalian species.

2. Patient and methods

2.1. Patients

13 CDA patients presented with clinical features of CDA were enrolled in the Institute of Hematology & Blood Disease Hospital. Informed consent was obtained from all patients and 30 healthy controls (15 males and 15 females) from Chinese Mainland according to the Declaration of Helsinki. Biochemical data on all patients were collected from the Institute of Hematology & Blood Disease Hospital and are shown in Table 1. Light microscopy and electron microscopy (Fig. S1), along with other clinical symptoms (normocytic or macrocytic anemia,