Congenital dyserythropoietic anemia type I (CDA I): Molecular genetics, clinical appearance and prognosis based on long-term observation

Running head: Congenital dyserythropoietic anemia type I

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Abstract

Congenital dyserythropoietic anemia type I (CDA I) is a rare autosomal recessive disorder with ineffective erythropoiesis and iron overloading. More than 100 cases have been described, but with the exception of a report on a large Bedouin tribe, these reports include only small numbers of cases and no data on the lifetime evolution of the disease are available. Since 1967, we have been able to follow 21 cases from 19 families for up to 37 years. Twenty-one patients with a confirmed diagnosis of CDA I exhibited chronic macrocytic anemia of variable severity requiring regular red cell transfusions only in two individuals. Four developed gallstones before the age of 30. Fifteen out of sixteen cases alive at the time of analysis showed mutations of at least one allele from exons 6 to 28 within CDAN1. Iron overloading is to be expected in all patients. In nine patients iron depletion was started between the ages of 7 and 36 years. Splenectomy, which was performed in 7 patients, did not result in improvement of hemoglobin values. Five patients were treated with Interferon α-2a, and all responded with a rise in hemoglobin concentration of between 2.5 and 3.5 mg/ml starting within 4 weeks.
Introduction

The congenital dyserythropoietic anemias (CDA) comprise a group of rare hereditary disorders of erythropoiesis that is characterized by ineffective erythropoiesis as the predominant cause of anemia and by distinct morphological abnormalities of the majority of erythroblasts in the bone marrow. The term was first used by Crookston et al for cases later classified as CDA II and by Wendt and Heimpel for cases later classified as CDA I. In addition to the index cases, a pair of non-identical twins, we were able to find two unrelated cases with the CDA I phenotype, by reviewing material of patients with unclassified congenital anemias. Further families were described mainly from France and Great Britain. Seventyseven cases from 68 families have been published as case reports, including 9 out of 21 confirmed cases of CDAI collated in the German CDA registry. In addition, there are 70 more cases in the large Bedouin tribe described by Tamary et al. from Israel in 1996. CDA I is characterized by congenital anemia, ineffective erythropoiesis and characteristic morphological abnormalities on both light- and electron microscopy. The severity of anemia varies considerably within and between families. Nonhematological dysmorphologies may be present, particularly peripheral limb abnormalities. As in CDA II, the main complication is iron overloading independent of transfusions. In members of the Bedouin tribe, the CDAN1 gene was mapped to chromosome 15q15.1-3 and a group of scientists of Israel and Europe recently cloned the mutant gene and described different mutations in nine families of Arabian, Polynesian and European origin. The protein encoded by the CDAN1 gene was named codanin-1.

Clinical characteristics, complications and problems of therapy are contained in case reports only, except for studies on the founder population of 45 children or young adults from the four extended Bedouin tribes from Israel. Here we report epidemiological data, clinical manifestations and the course of the disease in 21 patients from 19 unrelated families followed up by one of the authors. Mutations of the CDAN1-gene were investigated in all 16 patients who were alive at the time of analysis.
Patients and methods

Patients

A total of 25 patients from 22 families originally notified as CDA I were collated in the German CDA Registry. Diagnosis of CDA I was based on evidence of congenital chronic anemia and/or hyperbilirubinemia, with normal or inadequately increased reticulocyte counts and distinct hyperplasia of erythropoietic precursors suggesting ineffective erythropoiesis. All cases were reviewed in detail at the time of analysis. In 21 patients from 19 families morphological abnormalities of red cell precursors (see results) were typical for CDA I (Table 1). The following analysis is based on data from these 21 patients.

Clinical data were obtained from the institutions and physicians responsible for the patients’ management from 1967 to 2004 and collated in the German CDA Registry. The study was approved by the ethical committee of the University of Ulm, Germany. Informed consent was obtained for additional blood or bone marrow samples taken for research purposes. A unique patient number was assigned to each individual using a code identifying the family, the subjects and their relatives. Family trees were constructed by Cyrillic 2.1.2 (Cherwell Scientific Publishing Ltd.). Cross sectional data of nine patients from eight families were published previously2 3 14 15 10 16.

Clinical and laboratory data

Data used to analyze the course of disease were retrieved by both retrospective search for patients’ charts and other sources and by prospective monitoring after the diagnosis had been made and the patient was reported to the registry. Observation times, defined as the interval between the first and the last set of laboratory tests, ranged from 6 to 46 years. At the time of analysis five patients had died at ages between 31 and 57 years, living patients were between 6.5 and 55 years old. The number of days from which laboratory data were obtained varied between 4 and 44. For presentation of relevant parameters, means of all representative data after the age of three months and before splenectomy or treatment with interferon-α or phlebotomy were used, if not otherwise stated. Data measured after transfusion or at the time of aplastic crisis or severe, unrelated illness such as neoplasia were excluded.
Splenomegaly was defined as a palpable spleen or a size greater than reference values according to age and weight on ultrasound. Laboratory procedures depended on the time period in which they were performed. Estimation of red cell survival followed standard procedures\textsuperscript{17}. Electron microscopy was performed as described elsewhere\textsuperscript{8}.

**Analysis of the CDA I gene on genomic DNA**

Genomic DNA was isolated by the QIAmp DNA Blood Mini Kit (QIAGEN) according to the manufacturers instructions. Coding sequences and the exon/intron boundaries of the CDA I gene were amplified using the Taq polymerase system (QIAGEN). PCR products were sequenced directly using the BigDye Terminator v1.1 Cycle Sequencing Kit and an ABI 3100 Sequencer (Applied Biosystems). Primers used for PCR and sequencing and PCR conditions are available on request (klaus.schwarz@medizin.uni-ulm.de).

**Statistics**

MS – Access 2000 was used as the data bank management system, and statistical analyses were performed using SAS version 8.2. Event rates were calculated according to the method of Kaplan-Meier and subgroups were compared by the log rank test. If not otherwise stated, the Spearman rank correlation coefficient was used to correlate laboratory values and the Wilcoxon signed rank test to compare paired data.

**Results**

**Epidemiology and inheritance**

Seventeen and four patients came from 15 and 4 families resident in Germany or Switzerland, respectively. One family was of Turkish and one of Yugoslavian origin. In 11 out of the 17 families of German or Swiss origin, ancestry could be traced back to at least three (maximum nine) generations without showing origin from regions outside Central or Northern Europe. Consanguinity of parents of the probands was detected in two families. Basic laboratory data are available from 26 first-degree relatives; there was no unexplained chronic anemia or hyperbilirubinemia, except in three affected siblings of probands, which is compatible with the autosomal recessive heredity mode of inheritance of CDA I. Six female and six male patients had eight and ten healthy children, respectively.
Diagnosis and clinical presentation

Anemia and/or jaundice were usually recognized in childhood or in young adults. The ages at first diagnosis of CDA I ranged from 0.1 to 45 (median 17.3) years (Figure 1). In two patients, the diagnosis was made immediately after birth. Prior incorrect diagnoses included congenital hemolytic anemia (11), pernicious anemia (5), iron deficiency (4) or thalassemia (3). Seven patients had additional congenital malformations such as a sixth toe and syndactyly (3), a ventricular septal defect (1), short stature (1) double kidneys (1) or hip dysplasia (1). None of the patients were mentally retarded. At the time of diagnosis the spleen was enlarged in 17 patients.

Laboratory data

Key laboratory data of the 21 patients with confirmed CDA I are shown in Table 1. The degree of anemia varied, with mean hemoglobin concentrations between 6.4 and 13.2 g/dL. These values were almost stable throughout adult life, with the exception of single low values during severe infections or at pregnancy. Most but not all cases showed mild to distinct macrocytosis. The blood smear always showed anisopoikilocytosis as described elsewhere, with basophilic stippled erythrocytes in all and Cabot rings in three cases. Absolute reticulocyte counts were normal or moderately increased. Hemoglobin concentration as well as MCVs were lower and relative and absolute reticulocyte counts were higher in children as compared with adults (Figure 2). This could be shown by linear regression of all data (not shown). There were no significant differences between female and male patients. However, when values were compared only in individual patients with data available from an age of less and more than 15 years the differences were not significant. Before splenectomy, WBC and platelet counts were in the normal range throughout.

Red cell survival measured in six patients was moderately shortened, with apparent half times of 16 to 25 days (normal, 25 to 35 days). Total serum bilirubin at the time of diagnosis was moderately increased in 90% of all cases. This was exclusively due to an increase of the indirectly reacting fraction. When follow-up data were included, all cases showed hyperbilirubinemia. Independent of the observed fluctuations, some patients had consistently higher levels than others. In all cases, serum haptoglobin was absent or below age-adjusted reference values.
**Morphology**

All patients had typical bone marrow findings as previously described\(^4, 8, 9, 19\). Bone marrow specimens obtained by aspiration and/or trephine biopsy invariably showed distinct hypercellularity due to erythroid hyperplasia. The ratio of erythropoietic to granulopoietic cells (E/G ratio) varied between 3 and 8, compared to a normal range of 0.2 to 1. All four major abnormalities were seen: megaloblastoid aberrations of chromatin structure, large polyploid cells with an irregularly shaped nuclear mass often consisting of two segments, double- or triple-nucleated erythroblasts with nuclei of different sizes and structure and pairs of predominantly mature cells connected by thin chromatin bridges in 1.1 to 3.7 % of all erythroblasts. Thirty to sixty percent of all erythroblasts were considered abnormal.

Electron microscopy performed in 9 cases showed nuclear changes with widening of nuclear pores and condensation of heterochromatin with spongy or “Swiss cheese” appearance and additional less specific changes as described earlier\(^4, 8, 20, 21\).

**Molecular genetics**

Peripheral blood samples were available from 16 patients who were alive at the time of analysis (no samples were available from the 5 deceased patients). Fifteen patients with confirmed diagnosis of CDA I showed mutations of at least one allele from exons 6 to 28 within \(CDAN1\) (Table 2) with all but one mutation being localized in exons 12-28. In all, 17 different disease associated mutations were identified: seven had been described previously\(^12, 35\), whereas ten novel mutations were discovered (E 28 del -10 to +31 nts; del 1902-1904, 3259 insT; 3138 insTT; E 21 +2nts insCCG; Gln1182Stop; Arg724Trp; Arg1064Gln; Gly749Cys; Phe359Leu). These novel mutations were not detected in 58 additional genomes (i.e. 116 \(CDAN1\) alleles), excluding polymorphism. Of the novel mutations, two were deletions, three were insertions, one mutation generated a premature stop codon and four were missense mutations. Two of the novel mutations were predicted to affect the splice machinery. As expected, mutations were identical in two pairs of siblings (kindreds 026 and 409), and in the two alleles of case 447/01, the daughter of first-degree cousins of Turkish origin, both of whom healthy heterozygotes (data not shown). Four additional parents of compound heterozygous patients analyzed exhibited \(CDAN1\) heterozygosity without any signs of CDA I. In six patients we were unable to
describe more than one mutation, with the unidentified mutations most likely being located either in the 5’ or 3’ UTR, in the promoter, in enhancer regions or in introns of CDANI. Results at the protein level are lacking due to the unavailability of an antibody. Quantification of codanin, as well as search for its function are crucial points to be resolved in the future. In independent kindreds, mutations Pro671Leu and Pro1129Leu were present each on three alleles. Interestingly, no homozygotes or compound heterozygotes for null-type mutations have been identified, supporting an earlier notion that codanin-1 may have a unique function and may be essential during development.

No mutations of the CDAN1 gene were detected in patient 303/01, who showed the definite phenotype of CDA I with 33 chromatin bridges in 1000 erythroblasts counted; interestingly, iron loading was less than in other patients with 50% transferrin saturation and normal serum ferritin at the age of 40. For this case, pathogenic mutations remain to be discovered either in the CDA I-gene or in a second disease locus.

Prognosis and course of disease

At the time of analysis the age of living patients was between 6 and 55 years. Five patients had died at ages between 31 and 56 years. All adults had completed at least eight years of elementary and middle school education and six had graduated from college or acquired a similar professional qualification. Except for one patient who maintained hemoglobin concentrations of more than 11 g/dL, adolescents felt that their fitness was diminished compared to their classmates, and adults complained of moderate fatigue that became more marked during minor infections or social stress. Major health problems that can be regarded as dependent on the CDA are shown in Table 3. Patient 026/01 had a paravertebral bulk of extramedullary hematopoiesis which was removed without complications, and foci of hemopoiesis were detected in a liver biopsy in case 406/01. Leg ulcers, if present, were bilateral, overlaying or proximal to the medial or lateral malleoli or both.

Moderate splenomegaly was present before or at diagnosis in 17 out of 21 patients. In the majority of the other patients, splenomegaly became apparent in the first three decades of life. Gallstones were found in 4 patients before the age of 30 and were
sometimes detected in childhood or adolescence (Figure 3). Cholecystectomy was performed in two siblings at the ages of 44 and 51 years.

Twenty out of 21 patients developed iron overloading, as ascertained by serum ferritin and transferrin saturation and/or invasive or noninvasive estimation of liver iron. Only data obtained before splenectomy or interferon therapy and before a first course of iron depletion were used. Probability of ferritin values of \( \geq 300 \) or \( \geq 1000 \) ng/mL dependent on age are shown in Figure 4. There were large inter-individual differences. Maximal increments were seen between the ages of 20 and 40 years, although only one adult patient received regular transfusions. No differences between males and females were seen (Log-rank, \( p=0.45 \)). Concentrations of more than 2000 ng/mL with maximal values up to 5000 ng/mL were observed in seven patients in whom the diagnosis was delayed (or unknown before 1967), or in patients not properly monitored or incompliant to iron depletion therapy.

At the time of analysis, five patients had died between the age of 31 and 57 years, with heart and liver disease reported as the cause of death in three (027/01, 076/01, 417/01), squamous carcinoma of the ear in one (413/01) and septicemia (028/01) in one splenectomized patient. Autopsy data available for two patients (027/01, 076/01) who died at the ages of 31 and 48 years, respectively, revealed severe hemochromatosis.

**Therapy**

Red-cell transfusions were given to 12 patients. 5 patients received one to ten transfusions in the first four years of life, but not thereafter. Four females were transfused during pregnancy in order to ensure hemoglobin concentrations above 8 g/dL. With the exception of one male who received 28 transfusions no adult patient was transfusion dependent.

Several patients had been treated with iron (7), a variety of vitamins (5) or with prednisone (1) without evidence of improvement. Five patients (026/01, 026/02, 177/01, 178/01, 406/01) were treated with Interferon \( \alpha-2a \) with a starting dose of 9 Million units per week, and in one patient this was later switched to pegylated Interferon \( \alpha-2a \) 50 \( \mu \)g per week. They all responded with a rise of hemoglobin concentrations between 2.5 and 3.5 g/ml, the increase starting within 4 weeks after
the first application. Hemoglobin concentrations fell to pretreatment levels in two patients who felt that side effects decreased their quality of life.

Splenectomy was performed on seven patients ranging in age from 9 to 28 years. Data before and after splenectomy are available in six of those patients. Transient increase of hemoglobin concentration was seen in two cases, but was not different before and after splenectomy for the group as a whole (Wilcoxon signed rank test, \( p = 0.44 \)). Improvement of quality of life was not documented. The sister of one patient (case not included in this series) died one month after splenectomy for “hemolytic anemia”. All splenectomized patients had long standing thrombocytosis with maximum levels between 450 and 830 (median 780) G/L \((10^9/\text{L})\). Two had splanchic or major deep venous thrombosis. Further increase of ferritin after splenectomy was documented in four of the splenectomized cases and all had to be treated with deferoxamine.

Altogether, nine patients were treated with deferoxamine and/or deferiprone in doses recommended for treatment of secondary hemochromatosis in thalassemia major\(^{22}\). Treatment was usually begun when plasma ferritin reached a serum concentration of 1000 ng/mL. Age at initiation of deferoxamine therapy ranged from 7 to 35 (median 21) years. Reduction of elevated serum ferritin concentrations was achieved in all patients, while normal ferritin concentrations \(< 300 \text{ ng/mL}\) were reached in all patients with satisfactory compliance. Two patients underwent phlebotomies of 200-300 mL every four to six weeks, with normalization of ferritin and transferrin iron saturation.

Discussion

CDA I is a rare, but well defined clinical entity that has been observed in many regions in the world, most cases having been reported from the Central and Western European countries, North Africa and the Near East\(^{4} \, 5, \, 23\). Descent from non-European populations could not be detected in pedigrees of 19 out of 21 families residing in Germany or Switzerland (Table 1), and there is no evidence of a higher incidence in the large Mediterranean immigrant populations living in these countries than in families of Central European ethnicity. However, since the diagnosis requires microscopical assessment of bone marrow erythroblasts by an experienced expert, the ascertainment rate probably depends on the access of patients with chronic anemia to qualified hematological diagnosis; therefore, many cases go undetected. Indirect evidence for this hypothesis is the large variation of the ages at which the diagnosis is made. The distribution of
patients’ age as shown in Figure 1, is similar to that of 50 out of 70 cases identified from the literature (data not shown), excluding the patients of the highly inbred Bedouin tribes, in which all members were systematically investigated\(^7\). This is also true for cases that were newly diagnosed after CDA I was recognized as an independent entity in the late sixties of the twentieth century.

Most adult patients with CDA I have moderate macrocytic anemia with rather stable hemoglobin concentrations between 8 and 11 g/mL. Median values in our patients correspond well with data reported by others\(^4, 13, 24\). Occasionally, anemia may be severe enough to require blood transfusions in infancy and childhood but not thereafter, and exceptional patients remain transfusion-dependent in later life\(^25\). As a newborn, one female had severe anemia and neonatal sepsis requiring multiple red cell transfusions and intensive care, but was able to maintain stable hemoglobin concentrations after the age of 4 years. Similar observations have been made by others\(^26, 27\), and 17 of 31 patients of Bedouin tribe members were symptomatic in the newborn period\(^28\). On the other hand, one patient (UPN 409/04) showed macrocytosis without anemia, whereas his sister had moderately low hemoglobin values between 10 and 12 g/mL documented for 30 years. Only three cases with borderline anemia were described previously\(^20, 29, 30\).

Diagnosis of CDA I is based on evidence of an initially unspecified congenital dyserythropoietic anemia\(^4, 23, 31\) and typical morphology of bone marrow erythroblasts\(^4, 8, 9, 19\). Evidence of ineffective erythropoiesis was based on absence of adequate reticulocytosis in all our patients, contrasting with evidence of increased hemoglobin turnover as shown by greatly increased cellularity of bone marrow due to distinct hyperplasia of erythroblasts, indirect hyperbilirubinemia and depletion of haptoglobin. In addition, ineffective erythropoiesis had been previously demonstrated by combined DNA – measurement and \(^3\)H-Thymidin labeling in two\(^32\) and by ferrokinetic analysis in three of our patients\(^3\) as well as in cases reported in the literature\(^33, 34\). All 21 patients showed the characteristic morphological changes of erythroblasts, confirmed in 9 cases by electron microscopy. Chromatin bridges, which are a hallmark of CDA I, may be seen in no less than 1% of erythroblasts, and at least 500 consecutive erythroblasts should be examined when searching for this abnormality\(^4\). In contrast to CDA II\(^31\), to date no features other than morphology have been available to establish the diagnosis. Dgany et al\(^12\) were the first to demonstrate that mutations of the condanin-1 gene first observed in the Bedouin families were also present in six
unrelated families with CDA I from Europe and in one each form Arabia and Polynesia, as well as in eight kindreds from France\textsuperscript{35}. The observation of mutations of the codanin-1 gene in 15 out of 16 patients from Germany and Switzerland confirms these results. In accordance with similar clinical features as observed by the lifelong follow-up of our patients, these results suggest that CDA I is more than a phenotypic entity, and that the presence of codanin-1 mutations strongly supports the diagnosis of CDA I. DNA was available from additional four of the 25 patients originally enrolled in the German CDA Registry but not confirmed by review, and no mutations could be detected. CDA I may be of variable severity, but this variability seems to reflect a spectrum rather than the presence of different subentities. In the small group of patients analyzed, no phenotype-genotype correlation could be established from our mutation analyses. Analysis of our cases according to the need for transfusions in childhood, degree of anemia or hyperbilirubinemia or kinetics of iron loading failed to reveal correlations to the exon in which the mutation was detected. Since clinical severity was different in two pairs of siblings, factors determining “normal” variability of erythropoiesis are more likely to determine clinical expression than the codanin-1 mutation per se.

Although many patients with CDA I are able to realize their social and professional goals, others may have relevant morbidity and if untreated may even die of sequels related to CDA I. As in CDA II\textsuperscript{31} gallstones were detected more frequently and at younger age than expected in the healthy population. Bulky paravertebral erythropoiesis may occur, requiring thoracotomy in one of our patients. Iron overloading has been recognized as a potential consequence in most but not all patients described as case reports or cross-sectional studies of groups of cases\textsuperscript{3, 13, 24, 36, 37} but our observations are the first to show that on long term follow up iron overloading with potential tissue damage is to be expected in almost all patients. Lifelong monitoring of serum ferritin is therefore mandatory, and iron depletion should be started if ferritin concentrations approach a level of 1000 ng/ml, or if there is other evidence for organ damage by secondary hemochromatosis.

In contrast to CDA II, splenectomy is not recommend as a standard procedure even in patients with marked anemia, although in exceptionally severe cases it may lessen the need for transfusions\textsuperscript{38}. Our observations are consistent with the only report describing long term follow up in one patient\textsuperscript{30}. As in CDA II\textsuperscript{31}, splenectomy does not prevent further iron overloading. Interferon-alpha was effective in five of our patients.
and in all cases reported\textsuperscript{5}. According to a recently published follow up of the first patient treated with interferon-\textgreek{a}--2\textgreek{a}\textsuperscript{39}, this treatment, beyond normalizing the hemoglobin concentration, seems also to normalize the up-regulated enteral iron uptake, responsible for the iron overloading in CDA as well as in other states with chronic ineffective erythropoiesis\textsuperscript{40}.

One successful allogeneic sibling bone marrow transplantation was reported \textsuperscript{41}. Like those with other rare anemias, patients with CDA I should be treated by hematologists in cooperation with specialized centers in order to recognize disease-specific complications and to avoid superfluous diagnostic procedures as well as potentially harmful therapies. Monitoring of iron status to prevent clinically relevant secondary hemochromatosis is essential. Collation of all cases in national or international registries should be attempted in order to establish evidence-based recommendations for the lifelong management of these patients and to define the indication for special therapeutic measures such as therapy with interferon-\textgreek{a} or allogeneic stem cell transplantation in exceptionally severe cases.

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Table 1. Survey of patients with CDA I from the German CDA registry.

<table>
<thead>
<tr>
<th>CDA UPN</th>
<th>Age</th>
<th>Sex</th>
<th>Ethnic</th>
<th>Hb (g/dL)</th>
<th>MCV (fL)</th>
<th>Retic.ab (10^9/L)</th>
<th>Bilirubin (μmol/L)</th>
<th>Max Ferritin (ng/mL)</th>
<th>Morphology</th>
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<tr>
<td>026/01</td>
<td>54</td>
<td>f</td>
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<td>8.94</td>
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<td>8.00</td>
<td>n.d.</td>
<td>n.d.</td>
<td>18.89</td>
<td>n.d.</td>
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<tr>
<td>414/01</td>
<td>38</td>
<td>m</td>
<td>DE</td>
<td>10.84</td>
<td>104.9</td>
<td>43.8</td>
<td>37.23</td>
<td>3300</td>
<td>LM</td>
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<td>417/01</td>
<td>51</td>
<td>f</td>
<td>DE</td>
<td>9.00</td>
<td>n.d.</td>
<td>45.0</td>
<td>23.04</td>
<td>1900</td>
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<td>103.7</td>
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<tr>
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<td>18</td>
<td>f</td>
<td>DE</td>
<td>11.40</td>
<td>99.0</td>
<td>n.d.</td>
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<td>460</td>
<td>LM</td>
<td>23.24</td>
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<td>447/01</td>
<td>6</td>
<td>f</td>
<td>TR</td>
<td>7.18</td>
<td>86.6</td>
<td>115.5</td>
<td>41.53</td>
<td>810</td>
<td>LM</td>
<td>26.26</td>
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</tr>
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<td>m</td>
<td>DE</td>
<td>10.70</td>
<td>100.3</td>
<td>90.4</td>
<td>46.00</td>
<td>800</td>
<td>LM, EM</td>
<td>14</td>
<td></td>
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<tr>
<td>498/01</td>
<td>28</td>
<td>f</td>
<td>DE</td>
<td>8.45</td>
<td>100.5</td>
<td>71.2</td>
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<td>960</td>
<td>LM, EM</td>
<td>19</td>
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<td>95.6</td>
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<td>LM</td>
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<tr>
<td>529/01</td>
<td>34</td>
<td>f</td>
<td>YU</td>
<td>10.05</td>
<td>n.d.</td>
<td>n.d.</td>
<td>46.50</td>
<td>2450</td>
<td>LM</td>
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</table>

LM light microscopy; EM electron microscopy. DE German; CH Swiss; TR Turkish; YU Yugoslavian. Values of hemoglobin concentration (Hb), mean cellular volume (MCV) and total serum bilirubin (Bilirubin) are means of all representative data after the age of 15 years (see methods). For case 447/01 who was 6 years at the time of analysis the mean of all values after three months are used. For ferritin, maximal values are given. n.d. no data before splenectomy were available.
Table 2. *CDAN1* mutations in 16 cases of CDA I.

<table>
<thead>
<tr>
<th>CDA UPN</th>
<th>Genotype</th>
<th>Exon(s)/Introns affected</th>
<th>Nucleotide Position</th>
<th>Consequence at the mRNA or amino acid level</th>
<th>Status of the mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>026/01</td>
<td>het</td>
<td>IVS 27-E 28</td>
<td>E 28 del -10 to +31 nts</td>
<td>splice acceptor deleted</td>
<td>n</td>
</tr>
<tr>
<td>026/02</td>
<td>het</td>
<td>IVS 27-E 28</td>
<td>E 28 del -10 to +31 nts</td>
<td>splice acceptor deleted</td>
<td>n</td>
</tr>
<tr>
<td>177/01</td>
<td>comhet</td>
<td>12, 24</td>
<td>A1910G 3259 insT</td>
<td>Asn598Ser frameshift</td>
<td>d</td>
</tr>
<tr>
<td>178/01</td>
<td>comhet</td>
<td>24, 26</td>
<td>3259 insT C3503T</td>
<td>frameshift Pro1129Leu</td>
<td>n</td>
</tr>
<tr>
<td>406/01</td>
<td>comhet</td>
<td>14, 23</td>
<td>C2287T 3138 insTT</td>
<td>Arg724Trp frameshift</td>
<td>n</td>
</tr>
<tr>
<td>409/01</td>
<td>comhet</td>
<td>12, 24, 27</td>
<td>del 1902-1904</td>
<td>del Glu596 Arg1064Gln Gln1182Stop</td>
<td>n</td>
</tr>
<tr>
<td>409/04</td>
<td>comhet</td>
<td>12, 24, 27</td>
<td>del 1902-1904</td>
<td>del Glu596 Arg1064Gln Gln1182Stop</td>
<td>n</td>
</tr>
<tr>
<td>414/01</td>
<td>het</td>
<td>14</td>
<td>C2129T</td>
<td>Pro671Leu</td>
<td>d</td>
</tr>
<tr>
<td>421/01</td>
<td>comhet</td>
<td>14, 15</td>
<td>C2129T G2362T</td>
<td>Pro671Leu Gly749Cys</td>
<td>n</td>
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<tr>
<td>446/01</td>
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<td>C3106T A3242T</td>
<td>Arg997Stop Asp1042Val</td>
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<td>447/01</td>
<td>hom</td>
<td>26</td>
<td>C3503T C3503T</td>
<td>Pro1129Leu Pro1129Leu</td>
<td>d</td>
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<td>het</td>
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<td>C2254T</td>
<td>Arg713Trp</td>
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<td>het</td>
<td>IVS 21</td>
<td>E 21 +2nts insCCG</td>
<td>splice donor affected</td>
<td>n</td>
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</table>

Genomic DNA of 16 confirmed CDA I cases was analyzed for exonic (including exon-intron boundaries) *CDAN1* mutations. Nucleotide positions are numbered according to accession number NM_138477. Amino acid assignment was according to NP_612486. None of the detected changes is described in the single nucleotide polymorphism database dbSNP (http://www.ncbi.nlm.nih.gov/SNP/index.html). The shaded mutation denotes the loss of an in-frame triplet, leading to the deletion of amino acid 596 without influence on the reading frame. Family analysis linked del Glu596 to Arg1064 Gln.

hom = homozygote; het = heterozygote; comhet = compound heterozygote; n = novel (this publication) mutation; d = described mutation in reference 12 or 35.
Table 3. Functional consequences of anemia or secondary hemochromatosis.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. patient with disorder/total no. patients</th>
<th>Age of first presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>4/21</td>
<td>29 - 31</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4/21</td>
<td>30-50</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2/20</td>
<td>36-39</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>2/20</td>
<td>40-50</td>
</tr>
<tr>
<td>Hypogonadism</td>
<td>1/18</td>
<td>37</td>
</tr>
<tr>
<td>Exramedullary bulk</td>
<td>1/21</td>
<td>32</td>
</tr>
<tr>
<td>Leg ulcers</td>
<td>4/21</td>
<td>18 –40</td>
</tr>
</tbody>
</table>
**Figure 1.** Age at diagnosis
Figure 2. Key laboratory data of 21 patients correlated to age
Figure 3. Splenomegaly and gallstones detection dependent on age
Figure 4. Risk of iron overloading dependent on age. The probability of reaching concentrations of 300 ng/mL and of 1000 ng/mL serum ferritin is shown. Only data before treatment with interferon or deferoxamine or phlebotomy were used.
Reference List


Legends to the figures

**Figure 1**  
Age at diagnosis

**Figure 2**  
Key laboratory data of 21 patients correlated to age

**Figure 3**  
Splenomegaly and gallstones detection dependent on age

**Figure 4**  
Risk of iron overloading dependent on age. The probability of reaching concentrations of 300 ng/mL and of 1000 ng/mL serum ferritin is shown.
Congenital dyserythropoietic anemia type I (CDA I): Molecular genetics, clinical appearance and prognosis based on long-term observation

Hermann Heimpel, Klaus Schwarz, Monika Ebnother, Jeroen Goede, Detlev Heydrich, Torsten Kamp, Lothar Plaumann, Bettina Rath, Jochen Roessler, Otto Schildknecht, Mathias Schmid, Walter Wuillemin, Beate Einsiedler, Rosi Leichtle, Hannah Tamary and Elisabeth Kohne