


To the editor:

Congenital dyserythropoietic anemia type II in human patients is not due to mutations in the erythroid anion exchanger 1

Type II congenital dyserythropoietic anemia (CDA-II or HEMPAS) is an autosomal recessive disorder, representing the most frequent form of congenital dyserythropoiesis.

Recently, the molecular basis of the retsina (ret) phenotype that resulted in zebrafish from mutations in the gene that encodes the erythroid anion exchanger 1 (AE1, also called SLC4A1) was reported.1 The high number of binucleated erythroblasts, the presence of “double membranes,” and the reduction in posttranslational glycosylation of AE1, observed in the ret fish, are all reminiscent of the human CDA-II. Since the gene responsible for CDA-II in humans has not yet been identified, it is highly relevant to ask whether it could be AE1.

In first approximation, this does not seem likely for several reasons. First, as the authors themselves note, in a majority of families with CDA-II that have been subjected to linkage analysis, the disease maps to 20q11.2,2 whereas AE1 maps to 17q21-q22. Second, complete inactivation of AE1 in mice1 and in cattle4 causes severe hemolytic anemia, but not the CDA-II phenotype. Third, human erythroid AE1 absence has been found to cause severe hereditary spherocytosis (HS) but, again, no CDA-II.5

In order to test whether AE1 mutations might be responsible for a subset of patients with CDA-II we have used the resources of an international registry6 in which 108 patients are enrolled. In 14 of these patients, belonging to 7 unrelated families, the disease was not linked to 20q11.2 (Figure 1A).7 Of these 14 patients, 6 had a particularly severe form of CDA-II (they were transfusion dependent from the age of 1 year); the remaining 8 patients had “typical” CDA-II. In 5 families we were able to exclude linkage to chromosome 17. In addition, we proceeded in all 14 patients to quantitate erythrocyte AE1 by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).5,9 Whereas in patients with HS the amount of AE1 was significantly reduced (73.90 ± 2.42% of controls, P < .0001), in patients with CDA-II the amount of AE1 was virtually normal (96.50 ± 2.07% of controls, P = .78). In terms of the possible pathophysiologic significance of AE1 in CDA-II, while all these patients had the underglycosylation of AE1 that is characteristic of this condition, we did not find any difference in AE1 levels between patients with severe CDA-II versus “typical” CDA-II (96.83 ± 2.48% versus 96.25 ± 1.83%, respectively; P = .621) (Figure 1B).10

Based on genetic analysis and on biochemical findings, we suggest that in at least the vast majority of cases human CDA-II is not due to AE1 mutations. It is intriguing that, in zebrafish, AE1, through its protein 4.1R-binding domain, was found to affect the poles of the mitotic spindle specifically in erythroid cells; as a result, AE1 deficiency ultimately affects the completion of chromosome segregation in these cells, thus mimicking certain morphologic features of CDA-II. Therefore, in the rare patients in whom linkage to AE1 has not been ruled out, it will be important to look specifically for mutations within this particular domain of human AE1. At any rate, since these cases
are a minority, there must be in humans at least one other gene involved in this pathway.

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References


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Down-modulation of the C/EBPα transcription factor in core binding factor acute myeloid leukemias

Acute myeloid leukemia (AML) is characterized by abnormalities frequently affecting transcriptional control elements, leading to differentiation block. C/EBPα is one of the most frequently involved transcription factors, being disrupted by point mutations in 7% to 11% of cases. In the setting of the frequently involved transcription factors, being disrupted by the C/EBPα pathway was reported as a peculiarity of t(8;21)-positive AML, myeloid leukemias.

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Using a quantitative real-time polymerase chain reaction (RQ-PCR) method, we analyzed the C/EBPα expression levels in 144 bone marrows (BMs) from AML patients at diagnosis (French-American-British [FAB] distribution: M0, 15; M1, 19; M2, 38; M3, 22; M4, 37; M5, 9; and M6, 4) and in 32 samples (14 BM and 18 peripheral blood [PB]) from healthy controls. Following karyotypic and molecular analysis, 19 of 38 FAB M2 patients were classified as t(8;21) positive and 16 of 37 FAB M4 cases as inv(16) positive. C/EBPα expression was also tested in serial samples from 18 patients during follow-up (7 characterized by t(8;21), 5 by inv(16), and 6 by a normal karyotype).

As shown in Figure 1A, we detected a significant down-modulation of C/EBPα transcript in the samples positive for the presence of the t(8;21) and inv(16) cytogenetic abnormalities with respect to the other types of AMLs (mean value of C/EBPα copies/10^4 ABL copies: 33 638 vs 77 813, P = .0001), which showed values similar to those found in healthy controls (77 865 and 56 845 C/EBPα copies/10^4 ABL copies in normal BM and PB, respectively). Moreover, the comparison between the C/EBPα values obtained in BM samples from patients with AML1/ETO-positive FAB M2 AML compared with AML1/ETO-negative FAB M2 cases demonstrated a highly significant difference (32 523 vs 71 983 C/EBPα copies/10^4 ABL copies, P = .0001). Similar differences were detected by analyzing FAB M4 cases with and without the CBFB/MYH11 fusion (35 644 vs 105 173 C/EBPα copies/10^4 ABL copies, P = .0002). The Western blot assays further confirmed these


Figure 1. C/EBPα expression in the different FAB subtypes of AML and during follow-up. (A) C/EBPα expression is significantly downmodulated in FAB M2 AML patients characterized by the t(8;21) and in FAB M4 characterized by inv(16) with respect to those with normal karyotype. The mean values are defined by horizontal bars. (B) C/EBPα expression is significantly downmodulated in CBF AML patients at diagnosis, and it is up-regulated during chemotherapy-induced complete remission (CR). In the 8 CBF AML patients who persisted in CR (blue lines), C/EBPα expression did not change during follow-up; by contrast in the 4 CBF AML patients who relapsed (red lines), C/EBPα expression was downmodulated again. By contrast, no differences in C/EBPα expression were noted during follow-up in the 6 patients characterized by normal karyotype (green lines). Of these 6 patients, 4 persisted in CR and 2 relapsed.