Abnormalities of erythrocyte glycoconjugates are identical in two families with congenital dyserythropoietic anemia type II with different chromosomal localizations of the disease gene

We analyzed erythrocyte glycoconjugates in two families with congenital dyserythropoietic anemia type II (CDA-II): family 2 with the typical localization of the disease gene to chromosome 20q11.2 and family 1 in which this localization was excluded. Despite the different genetics, the erythrocyte glycoconjugate abnormalities in the two families were identical suggesting a complex inheritance of CDA-II. We also found that erythrocyte anion exchanger 1 protein is decreased in CDA-II homozygotes and obligate carriers alike.

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Congenital dyserythropoietic anemia type II (CDA-II) is a rare recessive disease characterized by ineffective erythropoiesis, the presence of bi-nucleated erythroblasts in the bone marrow, and a number of abnormalities affecting glycosylation and/or levels of erythrocyte glycoconjugates.[1-3] The abnormalities include hypoglycosylation of erythrocyte anion exchanger 1 (AE1), polyglycosylceramides (PGC), partial unglycosylation of glycophorin A[4] and elevation of contents of certain glycosphingolipids. Linkage analysis by means of microsatellite markers has shown that in most cases the disease gene *CDAN2* maps to 20q11.2[5] although in several families from southern Italy the gene is understood to have a different, but as yet undetermined, chromosomal localization.[6] Erythrocyte glycoconjugates in subjects with the latter sub-type of CDA-II have not been studied in detail. We compared the abnormalities of erythrocyte glycoconjugates in patients and obligate heterozygotes from two unrelated families, each with two children, in which the chromosomal localization of *CDAN2* differed. The localization of *CDAN2* was typical (20q11.2) in family 1 but unknown in family 2. The issue is not only of theoretical interest but also of practical relevance since analysis of erythrocyte glycoconjugates can be used for the detection of CDA-II carrier status.[7]

Tables 1 and 2 show that the abnormalities of erythrocyte glycoconjugates of the affected children and parents from families 1 and 2, when compared pair to pair, were quite similar. This was true for hypoglycosylation of polylactosamino-type glycans of AE1 (Table 1) and polyglycosylceramides (Table 2) as well as for partial non-glycosylation of O-linked glycans of glycophorin A (see Table 1 for its deficit in N-aetylgalactosamine, galactose and sialic acid residues). The accumulation of carbohydrates of oligoglycosylceramides was also identical in members of the two families (Table 2). Thus, mutations in genes that localize to different chromosomes[5,6] result in identical glycoconjugate abnormalities in erythrocytes. This suggests that the genes in question either regulate different events on a common pathway or co-operate in a single event that is essential for normal karyo-/cytokinesis of erythroblasts. It is not known, however, whether glycoconjugate abnormalities *per se* cause CDA-II[3] or whether they are a consequence of the disease.[8]

In keeping with previously obtained results[7] glycoconjugate abnormalities in erythrocytes from the parents of both families were intermediate between those of the affected children and healthy subjects. The only exception was the erythrocyte content of PGC in the parents, which contrary to findings of the previous study, was not elevated (*data not shown*). Nevertheless, a dosage effect was evident in the parents in four out of the five glycoconjugate indices examined. This finding makes the detection of CDA-II carrier state possible in heterozygotes through analysis of erythrocyte glycoconjugates also in patients with CDA-II subtype with the different genetic background .

Recently, evidence has been presented that a retsina mutation affecting the cytoplasm domain of AE1 in the zebrafish results in maturation arrest at the late erythroblast stage, and the presence of bi-nucleated erythroid cells.[9] Thus, in the present study we measured the erythrocyte content of AE1 protein, employing two different quantification techniques. With one technique we determined AE1 using flow cytometry of erythrocytes pretreated with eosin-5-maleimide[10] and found that the level of this protein was significantly reduced, on average by

Table 1. Erythrocyte membrane glycoproteins, AE1 and glycophorin A (GPA).

	Fuc		*GalNAc*		*GlcNAc*		*Gal*		*Man*		*NeuAc*		*Total CHO*	
							mol/mol							
Family	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Father	2.3	2.3	1.1	0.4	11.7	11.6	10.5	9.3	3.3	2.7	1.4	1.8	30.1	28.0
Mother	2.3	1.8	0.3	0.1	12.8	8.1	10.0	6.5	2.7	3.1	1.9	1.8	30.0	21.4
Child 1 AE1	1.2	1.1	1.4	1.0	5.0	6.1	4.4	4.8	3.4	3.9	1.3	2.0	16.7	18.8
Child 2	2.0	1.5	1.2	0.7	5.1	4.3	5.7	3.6	2.8	3.0	1.4	2.4	18.3	15.6
control (n=9)	2.4		1.2		14.7		14.1		2.8		0.8		35.9	
SD ±	0.5		0.5		0.8		1.4		0.3		0.5		1.1	
Father	1.3	2.7	9.4	9.1	5.2	4.9	17.5	15.0	2.8	2.7	17.4	17.4	53.6	51.8
Mother	1.8	2.8	7.9	7.7	5.2	4.7	17.6	15.6	3.0	2.8	17.3	15.6	52.8	49.2
Child 1 GPA	1.4	1.4	8.4	7.6	4.6	4.0	14.3	13.6	3.0	2.9	14.3	13.6	45.9	43.2
Child 2	1.5	1.9	7.0	7.8	4.9	4.3	14.9	14.3	3.1	3.0	14.5	14.3	45.8	45.5
control (n=9)	2.9		12.2		6.8		23.2		3.0		21.2		69.2	
SD ±	0.6		1.2		0.8		1.1		0.2		1.2		1.8	

For our methods see Zdebska et al.[7]. SD: standard deviation; CHO: carbohydrates.

Table 2. Erythrocyte membrane glycosphingolipids, oligoglycosylceramides and polyglycosylceramides (PGC).

	Carbohydrates of oligoglycosylceramides nmole/10^8RBC										CMC of PGC Total CHO mol/mol	
	Fuc		GalNAc		GlcNAc		Gal		Glc			
Family	1	2	1	2	1	2	1	2	1	2	1	2
Father	0.04	0.02	0.55	0.65	0.19	0.11	1.29	1.38	0.75	0.61	25.0	24.0
Mother	0.04	0.05	1.09	0.67	0.17	0.18	2.21	1.60	0.66	0.43	20.0	24.0
Child 1	0.12	0.16	1.27	1.56	1.07	1.21	4.62	3.96	1.48	2.12	16.0	13.0
Child 2	0.13	0.13	1.59	0.93	1.14	0.56	4.66	2.50	2.59	0.98	14.0	18.0
control (n=10)	0.02		0.42		0.08		0.86		0.32 (n=9)		36.7	
SD ±	0.01		1.10		0.03		0.21		0.08		2.6	

For methods see Zdebska et al.[7] Oligoglycosylceramides were, however, not fractionated but analyzed as a whole. CMC, carbohydrate molar composition; CHO: carbohydrates.

17.5%, in both the children and the parents (*data not shown*). The results were confirmed by electrophoretic determination of AE1, by which the mean reduction of AE1 content was 12.5% (*data not shown*). The results obtained by the two methods are in close agreement yet are not identical, probably because of an incomplete specificity of the flow cytometric method. This is caused by the reaction of eosin-5-maleimide with exofacial sulfhydryl groups of erythrocytes with about one fifth of the fluorescence intensity contributed by AE1.[10] A dosage effect in the parents was not observed. The reduction of AE1 in CDA-II erythrocytes might be associated with some abnormality of the AE1 protein, in line with the model of retsina mutation in the zebrafish.[9] A simpler explanation may, however, be a partial degradation of an improperly glycosylated AE1 in the erythroid cells by the quality control mechanism of the endoplasmic reticulum.

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