Disorders of Erythropoiesis

ET-1 and *ecNOS* gene polymorphisms and susceptibility to acute chest syndrome and painful vaso-occlusive crises in children with sickle cell anemia

The association of endothelin 1 (*ET-1*) and endothelial constitutive nitric oxide synthase (*ecNOS*) gene polymorphisms (G5665T and T8002C, VNTR and T-786C respectively) with the occurrence of acute chest syndrome and painful vaso-occlusive crises was evaluated in homozygous SS children. This retrospective study reveals that *ET-1* T8002 and *ecNOS* C-786 alleles are associated with, respectively, an increased and a decreased risk of acute chest syndrome.

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Acute chest syndrome (ACS) and painful vaso-occlusive crises (VOC) are the most common causes of hospitalization in sickle cell anemia and several studies have suggested the involvement of ET-1 and *ec*NOS in the pathogenesis of these two sickle cell anemia-specific complications.[1-4] In this study, we investigated the association of *ET-1* and *ecNOS* gene polymorphisms with the susceptibility to ACS and VOC in children with sickle cell anemia.

The polymorphisms studied were *ET-1* G5665T and T8002C variants, a variable number of tandem (27-base pair) repeats (VNTR) in intron 4 of the *ecNOS* gene and the *ecNOS* T-786C variant. Both *ET-1* variants have been associated with abnormal vascular reactivity.[5,6] The *ecNOS* VNTR is associated with the mean plasma nitric oxide level[7] while the *ecNOS* C-786 variant reduces *ecNOS* gene promoter activity and has been described as a genetic risk factor for ACS in adult female sickle cell anemia patients.[8]

Polymorphisms were analyzed in 173 SS children diagnosed at birth by systematic neonatal screening and followed longitudinally by the sickle cell center of Guadeloupe, with a mean follow-up period of 7.8±4.5 years. Due to the limitation of available DNA, genotype data for *ecNOS* T-786C, *ecNOS* VNTR, *ET-1* G5665T and *ET-1* T8002C loci were obtained for 159, 168, 171 and 157 patients, respectively.

VOC was defined as pain in the extremities, back and abdomen without an other explanation and excluding dactylitis. ACS was defined as the presence of new pulmonary infiltrates on a chest X-ray with chest pain and respiratory anomalies.[9]

Genotype frequencies were compared between children with and without ACS (ACS^+ and ACS^-), and with and without VOC (VOC^+ and VOC^-) using Pearson's test. Only genotype distributions for *ET-1* T8002C and *ecNOS* T-786C alleles were significantly different between ACS^+ and ACS^- groups of patients (p=0.039 and p=0.021, respectively) without difference between groups for age and fetal hemoglobin level (Table 1). As shown in Table 1, the incidence of *ET-1* C8002 was significantly higher in the ACS^+ group than in the ACS^- group, when the effect of the abnormal allele was assumed to be recessive (OD=5.6, 95% CI: 1.3 to 25.5, p=0.0164). In contrast, a lower incidence of *ecNOS* C-786 was observed in ACS^+ than in ACS^- patients, when both additive (OD=0.46, 95% CI: 0.25 to 0.86, p=0.0153) and dominant (OD=0.47, 95% CI: 0.21 to 0.84, p=0.018) effects of the mutant allele were assumed. Patients were stratified according to genotype and then frequencies of ACS, defined as the number of episodes per number of person-years of observation, were compared using the Mann-Whitney rank sum test. Concerning the *ET-1* T8002C polymorphism, the frequency of ACS was higher in patients with the CC genotype than in patients with the TC and TT genotypes (0.28±0.22 *vs.* respectively 0.18±0.20 and 0.14±0.22, p=0.038; Table 2). For the *ecNOS* T-786C polymorphism, patients carrying the CC and TC genotypes had lower frequencies of ACS than did patients with the TT genotype (respectively 0.09±0.15 and 0.15±0.19 *vs.* 0.29±0.21, p=0.033; Table 2). No gender effect was observed. We failed to find any association between the four polymorphisms and VOC (*data not shown*), a result potentially related to the statistical power of the present study, which ranged from 12% to 26% (α=0.05).

The *ET-1* C8002 allele appears to increase the risk of ACS and provides a basis for further investigations focusing on *ET-1* gene polymorphisms in the clinical variability associated with sickle cell anemia. Surprisingly, our findings suggest that the *ecNOS* C-786 allele decreases the risk of ACS in pediatric patients, contrasting with the

Table 1. Patients' characteristics and genotype frequencies of *ecNOS* and *ET-1* single nucleotide polymorphisms in patients stratified according to the occurrence of ACS.

SNP	*ACS+*	*ACS-*	*p*	*Odds ratios (95% CI)*
ET-1 T8002C				
Number of patients	95	62		–
Age (years)	11.2±4.2	10.6±4.8	NS	–
Sex ratio (M/F)	60/35	38/24	NS	–
HbF (%)	15.1±7.9	17.9±7.9	NS	–
Genotype count (frequency)				
CC	15 (.16)	2 (.03)		–
TC	24 (.25)	24 (.39)	0.021	–
TT	56 (.59)	36 (.58)		–
Additive effect	–	–	NS	1.36 (0.8-2.3)
Dominant effect	–	–	NS	0.96 (0.5-1.8)
Recessive effect	–	–	0.016	5.6 (1.3-25.5)
ecNOS T-786C				
Number of patients	104	55	–	–
Age (years)	11.2±3.1	9.9±4.8	NS	–
Sex ratio (M/F)	67/37	32/23	NS	–
Hb F (%)	15.9±8.2	17.9±8.8	NS	–
Genotype count (frequency)				
CC	1 (.01)	2 (.04)	–	–
TC	23 (.22)	21 (.38)	0.039	–
TT	80 (.77)	32 (.58)	–	–
Additive effect	–	–	0.015	0.46 (0.25-0.86)
Dominant effect	–	–	0.018	0.47 (0.21-0.84)
Recessive effect	–	–	NS	0.27 (0.02-2.9)

Patients were stratified according to the occurrence of ACS. Age and HbF level were compared using the Mann-Whitney rank sum test. Comparisons of the sex ratio and the ET-1 T8002C and ecNOS T-786C genotype frequencies were performed by Pearson's test. Odds ratios were calculated as an index of the association of ET-1 T8002C and ecNOS T-786C genotypes with the occurrence of ACS, with the effects of the abnormal alleles assumed to be additive (abnormal allele vs. normal allele), dominant (abnormal homozygote and heterozygote combined vs. normal homozygote) and recessive (abnormal homozygote vs. heterozygote and normal homozygote combined). NS: not significant.

Table 2. Patients' characteristics and frequency of ACS in patients stratified according to the genotype of *ET-1* and *ecNOS* polymorphisms.

	ET-1 T8002C			*p*	*ecNOS T-786C*			*p*
	CC	*TC*	*TT*		*CC*	*TC*	*TT*	
N	17	48	92	–	3	44	112	–
Age	11.4±5	10.3±4.8	9.8±4.8	NS	14.7±2.3	9.6±4.2	10±5	NS
Sex ratio (M/F)	9/8	35/13	54/38	NS	3/0	27/17	69/43	NS
HbF (%)	12.9±8.1	18±8.5	16.1±7.9	NS	15.6±9.8	16.8±8.2	16.7±8.7	NS
ACSn	0.28±0.22	0.18±0.20	0.14±0.22	0.038	0.09±0.15	0.15±0.19	0.29±0.21	0.033

Patients were stratified according to the genotype of ET-1 T8002 and ecNOS T-786C polymorphisms. Age, HbF level and the frequencies of ACS were compared using the Kruskal-Wallis test. Comparison of the sex ratio was performed by Pearson's test. n : frequency of ACS defined as the number of episodes per number of person-years of observation. NS: not significant.

results of a previous study which had shown that this allele is associated with a higher risk of ACS but only in adult female patients. These two studies are not, however, comparable because of the age-related differences in the etiology, the clinical picture and the course of ACS.[10] In children with sickle cell anemia, ACS is commonly associated with infection while in adult patients, other clinical events such as bone marrow fat emboli are considered to play a significant pathogenic role in ACS. The conflicting results could imply that consequences of nitric oxide deficiency in sickle cell anemia patients carrying the *ecNOS* C-786 differed accordingly to the events triggering the ACS. Further studies are warranted, in other sickle cell anemia populations, to confirm these findings.

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Key words: sickle cell anemia, ET-1, ecNOS, polymorphisms, acute chest syndrome, painful crises.

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References

1. Graido-Gonzalez E, Doherty JC, Bergreen EW, Organ G, Telfer M, McMillen MA. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. Blood 1998;92:2551-5.
2. Hammerman SI, Kourembanas S, Conca TJ, Tucci M, Brauer M, Farber HW. Endothelin-1 production during the acute chest syndrome in sickle cell disease. Am J Respir Crit Care Med 1997;156:280-5.
3. Morris CR, Kuypers FA, Larkin S, Vichinsky EP, Styles LA. Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. J Pediatr Hematol Oncol 2000;22:515-20.
4. Hammerman SI, Klings ES, Hendra KP, Upchurch GR Jr, Rishikof DC, Loscalzo J, et al. Endothelial cell nitric oxide production in acute chest syndrome. Am J Physiol 1999; 277: H1579-92.
5. Iglarz M, Benessiano J, Philip I, Vuillaumier-Barrot S, Lasocki S, Hvass U, et al. Preproendothelin-1 gene polymorphism is related to a change in vascular reactivity in the human mammary artery in vitro. Hypertension 2002;39:209-13.
6. Kozak M, Izakovicova Holla L, Krivan L, Vaskuo A, Sepsi M, Semrad B, et al. Endothelin-1 gene polymorphism in patients with malignant arrhythmias. J Cardiovasc Pharmacol 2004; 44:S92-S95.
7. Tsukada T, Yokoyama K, Arai T, Takemoto F, Hara S, Yamada A, et al. Evidence of association of the ecNOS gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 1998;245:190-3.
8. Sharan K, Surrey S, Ballas S, Borowski M, Devoto M, Wang KF, et al. Association of T-786C eNOS gene polymorphism with increased susceptibility to acute chest syndrome in females with sickle cell disease. Br J Haematol 2004;124:240-3.
9. Tarer V, Etienne-Julan M, Diara JP, Belloy MS, Mukizi-Mukaza M, Elion J, et al. Sickle cell anemia in Guadeloupean children: pattern and prevalence of acute clinical events. Eur J Haematol 2006;76:193-9.
10. Vichinsky EP, Styles LA, Colangelo LH, Wright EC, Castro O, Nickerson B. Acute chest syndrome in sickle cell disease: clinical presentation and course. Cooperative Study of Sickle Cell Disease. Blood 1997;89:1787-92.