Association of HbS and a new low oxygen affinity variant, Hb Canebière, [β102(G4)Asn->Lys] in a healthy child

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A 5-month-old boy was admitted to hospital for an episode of bronchiolitis. Despite the rapid resolution of his pulmonary symptoms, his oxygen saturation value remained low (SpO2: 82%) as monitored with a pulse oxymeter. This value was normalized by oxygenotherapy but diminished again when oxygenotherapy was stopped. Clinical examination, blood count and red cell indices were normal, as were chest x-ray and echocardiography. A sample of arterial blood at room temperature was analysed for oxygen saturation using co-oxymetry, and showed a normal partial oxygen pressure, a decreased SaO2 (83%), and an increased proportion of deoxyhaemoglobin (16%). Methaemoglobin and carboxyhaemoglobin values were normal. Measurement of the whole blood P50 revealed an exceptionally high value of 54mmHg (normal value: 26±1), suggesting the possible presence of a low oxygen affinity haemoglobin variant. The child was known to have a sickle cell trait, detected at birth by neonatal screening, but heterozygosity for HbS cannot explain the observed P50 value. Chromatography analysis performed at age 2 months in the course of neonatal screening control (Variant Sickle cell short program, Hercules CA), showed HbF, HbS and an HbX peak, which was initially thought to be HbA (retention time: 1.03 min.). The mother, who originates from Tunisia, is heterozygous for HbS and the father's chromatography profile is normal. Because of the high P50 value, we performed haemoglobin analyses again in the proband (at the age of 5month), including HPLC and electrophoresis. Chromatography analysis, confirmed by cellulose acetate electrophoresis, showed an HbS fraction (38%), HbF (9.6%) and an unidentified fraction of 48.8% (retention time 1.13 min.). Molecular analysis of the proband's β-globin gene revealed an A to C substitution in exon 2 leading to the replacement of Asn by His at codon 102 (AAC to CAC). This variant, which results from a de novo mutation, has never been described in the literature and was named Hb Canebiere. Purified Hb Canebiere presents a low oxygen affinity (Table 1a) and, while cooperativity has been conserved, it is decreased. The Bohr effect and the chloride effect were, respectively, 30% and 50% less than normal (Table 1b).

Three other variants with substitution at the codon 102 have been reported so far, and they all lead to low oxygen affinity haemoglobins due to destabilization of the R conformation.[1-3] Nagel *et al.* have suggested a correlation between the size of the substituting amino acid side chain and the level of oxygen affinity.[4] As regards side chain size, Hb Canebière ranks between Hb Saint Mandé and Hb Kansas, and this is also the case for the P50 value.

In the case reported here, the association with Hb S lowers the global O2 affinity, leading to one of the lowest observed in vivo, but it does not promote polymerisation of HbS. The patient is now 3 years old and has no clinical or biological symptoms, except transient cyanosis of the lips, confirming that low-affinity variants are clinically well tolerated. The frequency of the sickle cell trait or β-thalassemia trait has increased over the last 50 years in Northern Europe because of population migration from countries endemic for haemoglobinopathies. These traits are sometimes associated with extremely rare haemoglobin variants, which are usually asymptomatic in the heterozygous state, but whose properties can be exaggerated when HbA is absent or replaced by HbS. The clinical outcome can theoretically be predicted according to the physical properties of the variant but case reports, if available, are helpful for setting up an appropriate clinical follow up or providing consistent genetic counselling for the parents.

Table 1. a: Oxygen binding properties of purified Hb Canebière

	Hb A		*Hb Canebière*		P_{50} *Hb A* / P_{50} *Hb Canebière*
Experimental conditions	P_{50} (torr)	n_{50}	P_{50} (torr)	n_{50}	
pH 7.2 [NaCl] 0.1M	4.8	2.7	11.7	1.9	0.41
pH 7.2 [NaCl]=0	1.3	1.8	4.0	1.9	0.33
pH 7.2 [NaCl] 0.2M	7.4	2.7	14.2	1.8	0.52
pH 7.2 [NaCl] 0.1M +DPG = 1 mM	14.0	2.8	35.9	1.7	0.39
pH 6.5 [NaCl] 0.1M	11.0	2.6	19.5	1.7	0.56

Other conditions: bis-Tris 0.05 M, EDTA 50 μM, catalase 20 μg/mL, [heme] = 50-60 μM, 25°C.
% MetHb = 3 to 5 % after each OEC.

b: Heterotropic effects of purified Hb Canebière.

	Hb A	*Hb Canebière*
Chloride effect (Δlog P50 /Δlog [NaCl]) :	+ 0.63	+ 0.28
Bohr effect (Δlog P50 /ΔpH) :	- 0.51	- 0.49
DPG effect :(Δlog P_{50}±1 mM DPG)	+ 0.46	+ 0.32

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References

1. Arous N., Braconnier F., Thillet J., Blouquit Y., Galacteros F., Chevrier M., Bordahandy C., Rosa J.. Hemoglobin Saint Mande beta 102 (G4) asn replaced by tyr: a new low oxygen affinity variant. FEBS Lett. 1981; 126(1):114-6.
2. Bonaventura J and Riggs A. Hemoglobin Kansas, a human hemoglobin with neutral amino-acid substitution and an abnormal oxygen equilibrium. J. Biol. Chem. 1968, 243: 980.
3. Nagel R.L., Lynfield J., Johnson J., Landau L., Bookchin R.M., Harris M.B., Hemoglobin Beth Israel. A mutant causing clinically apparent cyanosis. N. Engl. J. Med. 1976; 295(3):125-30.
4. Nagel R.L. Disorders of hemoglobin function and stability, in : MH Steinberg, BG Forget, DR Higgs, RL Nagel, eds. Disorders of Hemoglobin, Genetics, Pathophysiology and Clinical management, 1st ed, Cambridge University Press, 2001: 1155-1194.