Neapolis (CD 126 β⁺ GTG→GGG): a result of a screening in Campania, a region in Southern Italy

Between January 1995 and December 2005, we conducted a screening program for the presence of Hb Neapolis, a rare abnormal Hb variant, in Campania, a region in Southern Italy. Nineteen patients with Hb Neapolis in heterozygosis and six patients with a genetic compound (Hb Neapolis/β-thalassemia) were identified. Patients with Hb Neapolis in heterozygosis showed a slight alteration in HbA_2 levels while compounds showed typical characteristics of thalassemia intermedia ranging from a non transfusion-dependent form for five patients to a transfusion-dependent form for one adult patient.

Haematologica 2007; 92:990-991

In 1990, a new Hb variant called Dhonburi where valine at residue 126 of the β-globin chain was replaced by glicine was first described. This Hb variant was found in association with β^0-thalassemia leading to a β-thalassemic intermedia phenotype.[1] In 1991, we found the same abnormal hemoglobin (called Neapolis) affecting three unrelated families from the Campania region (Southern Italy).[2] More recently, Hb Neapolis was described in Campania in combination with either Hb Lepore-Boston[3] or the δ-thalassemic defect δ^+27 (G→T).[4] Therefore, an ongoing screening program in our Thalassemic Unit to identify α and/or β-thalassemia traits was expanded to also search for the presence of Hb Neapolis. Between January 1995 and December 2005, approximately 30,000 healthy subjects were screened.

Red blood indices were measured by Cell-Dyn 3700 (Abbott USA), and hemoglobin analysis was performed by high performance liquid chromatography (HPLC) (Variant II, Bio-Rad Laboratories, Richmond, CA, USA). Hb Neapolis carrier status was suspected in the presence of moderate hematologic alterations: i.e. slight increase in HbA_2 (> 3.3%) and normal or decreased of MCV (<78fL). Compounds were suspected in the presence of moderate anemia with *HbA_2* >6.0% values and HbF >1.5%. HPLC did not allow a clear distinction to be made between Hb Neapolis and HbA_2. Therefore, heat stability and isopropanol precipitation tests were used as previously described.[5] Samples positive at previous tests were analyzed by PCR-ARMS (Polymerase Chain Reaction-Amplification Refractory Mutation System) or by DNA sequencing. Sequence analysis of exon 3 of β-gene was carried out using the following primers: nucleotides 63169-63192 and 63726-63744 (GenBank), and ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturers' instructions. Allele specific amplification analysis was obtained by PCR amplification of β gene CD 126 (GTG→GGG) using primers according to Pagano *et al.*[3] β-Thal mutations were identified by reverse hybridization assay (β-globin StripAssay, Nuclear Laser, Vienna Lab). The δ27 (G→T) mutation was detected by PCR-ARMS.[4] Haplotype analysis of DNA polymorphisms on β-globin gene cluster was performed as described by Orkin *et al.*[6]

Hb Neapolis (β126(H4) Val →Gly [GTG→GGG]) was identified in heterozygosis in nineteen subjects from ten families (group 1) and in six patients in association with a β-thalassemia mutation (group 2). All patients were from Campania. Some were from Naples. Group 1 patients' characteristics at diagnosis are shown in Table 1. They showed slight alterations in hematologic data. Some patients had mild anemia and average Hb concentration was 12.6 gr/dL (12.4±0.9 g/dL for females, 13.0±1.3 g/dL for men). All patients had slightly increased HbA_2 levels (average±SD=3.55±0.15%). A decrease in MCV values (average±SD=76.0±3.88 fL) was found in 9 out of 18 subjects. Other hematologic parameters and ferritin values of 57±40 ng/mL were in the normal range confirming the mild phenotype of Hb Neapolis carriers.

Group 2 patients' characteristics are shown in Table 2. All patients showed a hematologic phenotype of thalassemia intermedia. Five Hb Neapolis/CD39 patients had never been transfused or had only been transfused during pregnancy (patient *GD*) while one patient (*AMR*) with associated IVSII-1 was transfusion-dependent.

Table 1. Hematological and biochemical data of heterozigotes Hb Neapolis (group 1).

Subjects	*Age/ sex*	*RBC*	*Hb (g/dL)*	*MCV (fL)*	*MCH (pg)*	*HbA_2 %*	*HbF %*	*Ferritin ng/mL*	*Stability tests*
A.G.	13/F	5.6	13	79	24	3.5	1	12	+
D.P.A	56/F	4.4	12.5	80	27	3.3	1	120	+
A.A.	18/M	5.1	12.3	74	23	3.6	0.9	15	+
I.L.	39/F	4.2	11.3	79	26	3.5	0.9	45	+
E.P.C	11/M	5.4	13.3	75	24	3.3	0.8	19	+
E.P.S	15/M	5.0	12.0	70	24	3.8	0.9	24	+
E.P.G	12/F	5.6	13.6	72	24	3.6	0.9	96	+
M.A.	28/F	4.7	13.0	80	27	3.7	0.8	29	+
C.P.	49/M	5.4	13.4	75	25	3.4	0.9	98	+
C.T.	19/M	4.9	11.1	69	23	3.6	0.8	56	+
M.A.	35/M	5.9	14.8	77	25	3.4	0.9	98	+
M.L.	28/F	5.2	12.2	71	23	3.5	0.8	102	+
B.F.	31/F	3.9	10.3	78	26	3.6	0.9	9	+
D.A.	35/F	5.1	12.8	72	25	3.6	0.8	25	+
D.E.	17/F	4.4	11.9	79	27	3.7	0.9	19	+
D.S.	47/M	5.3	14.0	80	26	3.6	0.8	112	+
E.P.	32/F	4.6	12.3	78	27	3.5	0.9	55	+
D.G.	44/F	4.9	13.2	81	27	3.8	0.8	95	+
S.R.*	**19/F**	**5.2**	**12.9**	**71**	**24**	***2.7**	**0.8**	**52**	**+**
Average	**29**	**4.98**	**12.6**	**76**	**25**	**3.5**	**0.88**	**57**	
SD	**13.9**	**0.53**	**1.08**	**3.88**	**1.50**	**0.15**	**0.07**	**40.12**	

**S.R. was an Hb Neapolis and δ-thal compound; SD: standard deviation*

Table 2. Hematologic and biochemical data of compound Hb Neapolis/β thalassemia (group 2).

Patients	*Age/ sex*	*RBC*	*Hb (g/dL)*	*MCV (fL)*	*MCH (pg)*	*HbA_2%*	*HbF%*	*Ferritin ng/mL*	*β-thal mutations in trans of Hb Neapolis*
M.A.	7/F	4.5	8.9	57	19	6.5	2.0	194	CD 39
C.M.	5/M	5.6	9.7	59	18	6.1	14.0	97	CD 39
S.C.D.	9/M	6.1	9.8	57	17	6.9	8.0	80	CD 39
G.D.	22/F	4.7	9.3	59	19	7.0	3.0	425	CD 39
G.G.	24/M	5.4	9.6	58	19	7.2	7.0	416	CD 39
A.M.R.	50/F	5.6	7.8	58	15	6.8	1.5	1447	IVS-II-1

Data represent the mean of at least four samples per year since diagnosis.

Among the five transfusion independent patients Hb concentration was 9.46±0.36 g/dL, MCV was 58±1 fL, HbA_2 was 6.74±0.44% and HbF was 6.8±4.76%. A slight increase in serum ferritin levels 242±168 ng/mL was also observed. Spleen enlargement was presented in only three out of six subjects. All patients showed a slight liver enlargement. One patient underwent cholecystectomy because of gallstones and another had microlithiasis of the gallbladder (*data not shown*).

Finally, haplotype analysis of DNA polymorphisms on the β-globin gene cluster showed that all patients carried the same haplotype V (d*ata not shown*). This differs from the haplotype VII found in Hb Dhonburi in Thailand and agrees with the recent sugestion that two independent mutational events have taken place.[7]

Based on the results of this study, the overall incidence of Hb Neapolis in the screened population was almost 0.09%. This is similar to that observed in a report of Than *et al*. on populations in the Southern Shan state, Myanmar (ex-Burma).[8] In conclusion, results of our screening show that Hb Neapolis carriers were accurately identified among the Campania population. This should encourage wider screening. In fact, we confirm that compound Hb Neapolis/β^0-thalassemia presents characteristics of thalassemia intermedia even in transfusion-dependent patients. However, our series was limited to CD 39/and IVS-II/Hb Neapolis compounds and consequently their clinical phenotypes remain unpredictable. This makes it extremely difficult to provide accurate genetic counselling for this form of thalassemia.

Leonilde Pagano, Assunta Viola, Gennaro Fioretti, Massimiliano Ammirabile, Paolo Ricchi, Luciano Prossomariti

UOC Centro delle Microcitemie "A. Mastrobuoni", AORN A. Cardarelli Napoli, Italy

Key words: Hb Neapolis β 126 (H4) Val→Gly, β-thalassemia, thalassemia intermedia.

Correspondence: Leonilde Pagano, UOS, Diagnosi delle Talassemie, UOC di Microcitemia, Azienda Ospedaliera di Rilievo Nazionale "A. Cardarelli", Via A.Cardarelli 9, 80145 Naples, Italy. Phone: international +39.081.7472242. Fax: international +39.081.7472248. E-mail: ildepagano@libero.it

References

1. Bardakdjian-Michau J, Fucharoen S, Delanoe-Garin J, Kister J, Lacombe C, Winichagoon P, et al. Hemoglobin Dhonburi α2,2 126(H4) Val→Gly: a new unstable , variant producing a β-thalassemia intermedia phenotype in association with β^0-thalassemia. Am J Hematol 1990;35:96-9.
2. Pagano L, Lacerra G, Camardella L, De Angioletti M, Fioretti G, Maglione G, et al. Hemoglobin Neapolis, β126(H4) Val→Gly: a novel β-chain variant associated with a mild β-thalassemia phenotype and displaying anomalous stability features. Blood 1991;78:3070-5.
3. Pagano L, Carbone V, Fioretti G, Viola A, Buffardi S, Rametta V, et al. Compound heterozygosity for Hb Lepore-Boston and Hb Neapolis (Dhonburi) [β126(H4) Val→Gly] in a patient from Naples, Italy. Hemoglobin 1997;21:1-15.
4. Grosso M, Rescigno G, Zevino C, Matarazzo M, Poggi V, Izzo P. A rare case of compound heterozygosity for δ(+)27 and Hb Neapolis (Dhonburi) associated to an atypical β-thalassemia phenotype. Haematologica 2001;86:985-6.
5. Huisman THJ, Jonxis JPH. The Hemoglobinopathies Techniques of Identification Clinical and Biochemical Analysis .Vol. 6 New York; Marcel Dekker, Inc. 1977
6. Orkin SH, Kazazian HH Jr, Antonarakis SE, Goff SC, Bohem CD, Sexton JP, et al. Linkage of β-thalassaemia mutations and β-globin gene polymorphism in human β-globin gene cluster. Nature 1982;296:627-31.
7. Viprakasit V, Chinchang W. Two independent origins of Hb Dhonburi (Neapolis)[β 126 (H4) Val→Gly]: An electrophoretically silent haemoglobin variant. Clin Chim Acta 2007;376:179-83.
8. Than AM, Harano T, Harano K, Myint AA, Ogino T, Okadaa S. High incidence of β-thalassemia, hemoglobin E, and Glucose-6-Phosphate dehydrogenase deficiency in populations of Malaria-endemic Southern Shan State, Myanmar. Int J Hematol 2005;182:119-23.