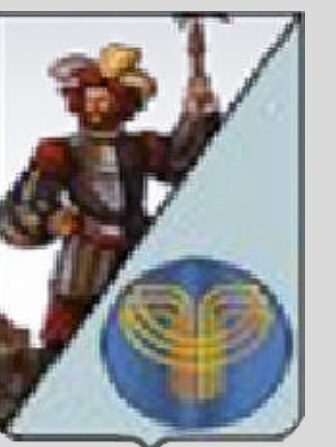




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THE GENETIC HETEROGENEITY OF BETA GLOBIN VARIANTS IN THE SICILIAN POPULATION

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INTRODUCTION

Hemoglobinopathies are autosomal recessive conditions affecting the expression (thalassemia) or the structure (abnormal hemoglobin) of the globin gene products. In Sicily, β -thalassemia is the most common inherited blood disorder as well as in several other Mediterranean countries. It's incidence was estimated about 6% while variant hemoglobin carriers account for 2% of whom Hb S is the most represented. The number of Hb variants identified in the world is steadily increasing (<http://globin.cse.psu.edu/globin/hbvar>), thanks to laboratory techniques more and more refined. Their prevalence varies considerably according to geographical location and ethnic group, while for some it is often underestimated because did not show any alteration of hematological and electrophoretic parameters and were occasionally identified during familiar molecular analysis for prevention of thalassemia. Migration flows make also the picture of the distribution of variants extremely varied. We present a retrospective study performed on 1330 carriers of Hb variants selected, during the last twenty years, from subjects coming to our Thalassemia Center in Palermo for a β -thalassemia trait test and from a school screening program. We identified 24 variants of the beta globin gene, most of which has been occasionally identified during family studies for familiar molecular analysis for prevention of thalassemia. The presence of the numerous structural Hb variants identified in the Sicilian population can be considered a further testimony of the presence of many civilizations in the island and contacts with people from Africa, Orient and Mediterranean origin.

MATERIALS and METHODS

The red cell indices were measured on an automated blood cell counter and HbA, HbA2, HbF and Hb variants, during the last 5 years, were identified and measured by HPLC on a TOSOH G7 and G8 system using β -thal mode. Patients affected with thalassemia major or intermedia or double heterozygote for variant hemoglobins and immigrant subjects were excluded. DNA was extracted from the buffy-coat using salting out precipitation. Allele-specific oligonucleotide hybridisation (ASO) with radioactive probes, restriction enzyme analysis of amplified product (RE), amplification refractory mutation system (ARMS), reverse dot blot (RDB) analysis were carried out for direct detection of the most common mutations present in the Sicilian population. Radioactive sequencing and successively automatic sequencing were used to screen rare or unknown mutations. Specific primers were used to identify deletional or recombinant defects. The Flow-Chart used for the identification of Variants hemoglobin is show in Fig.1.

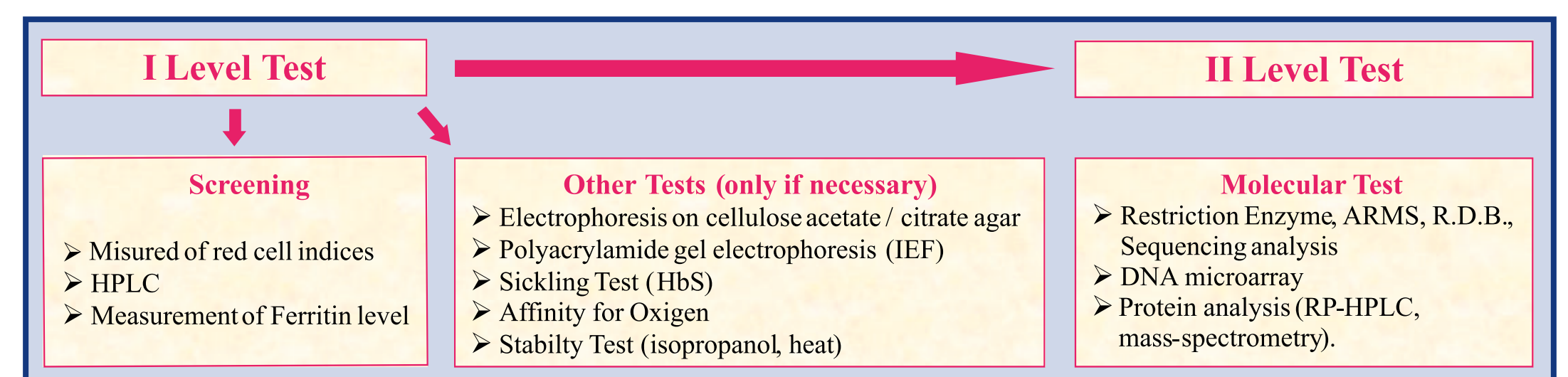


Fig. 1

RESULTS

Six were most common: Hb S (beta 6 (A3) Glu> Val), Hb C (beta 6 (A3) Glu> Lys), the Hb Lepore-Boston-Washington (from delta 87, in beta 116), Hb D-Los Angeles (beta 121 (GH4) Glu> Gln), Hb G-Copenhagen (beta 47 (CD6) Asp> Asn) and Hb G-San José (beta 7 (A4) Glu> Gly).

Hb S is the most representative hemoglobin variant (72,1%); its distribution is not uniform in the island: the bS allele has a higher location along the south-eastern coast, while in the western side is rarer.

Additionally it was found that in Gela, a village in the province of Caltanissetta founded by the Arabs, the Hb S was found with a percentage of 13%: the high frequency is probaly due to the fact that Gela, in past centuries, is was considered to be the main port of the south-east of Sicily for trade between the MedioOriente, the Mediterranean and north Africa.

In the north east of Sicily (Palermo) were identified 61 subjects (4.58%) carriers of Hb C, a variant that is more rare in other parts of the island. Hemoglobin Lepore-Boston-Washington is the third most common variant found, with a percentage of 4.28%, while the HbD-Los Angeles and HbG-Copenhagen were found respectively in 56 (4.21%) and 47 (3.53%) subjects with a higher incidence in the eastern side of Sicily rather than in the West. Tab. 1 shows variants identified in our laboratory and following are examples of some variants electrophoretic profiles.

Mutation	Frequency (%)	No. of cases
Hb S (beta 6(A3) Glu>Val)	72.1	958
Hb C (beta 6(A3) Glu>Lys)	4.58	61
Hb Lepore-Boston-Washington (delta through 87; beta from 116)	4.28	57
Hb D-Los Angeles (beta 121(GH4) Glu>Gln)	4.21	56
Hb G-Copenhagen (beta 47(CD6) Asp>Asn)	3.53	47
Hb G-San José (beta 7(A4) Glu>Gly)	2.55	34
Hb O-Arab (beta 121(GH4) Glu>Lys)	1.58	21
Hb Yaounde (beta 134(H12) Val>Ala)	1.05	14
Hb Beirut (beta 126(H4) Val>Ala)	0.75	10
Hb Camperdown (beta 104(G6) Arg>Ser)	0.75	10
Hb Valletta (beta 87(F3) Thr>Pro)	0.82	11
McKees Rocks (beta 145(HC2) Tyr>His--O)	0.52	7
Hb Marseille (beta 2(NA2) His>Pro)	0.45	6
Hb Athens-GA (beta 40(C6) Arg>Lys)	0.37	5
Hb Acharnes (beta 53(D4) Val>Thr)	0.37	5
Hb City of Hope (beta 69(E13) Gly>Ser)	0.37	5
Hb Ernz (beta 123(H1) Thr>Asn)	0.28	4
Hb Marinese (beta 70(E14) Ala>Val)	0.22	3
Hb Hershey (beta 70(E14) Ala>Gly)	0.22	3
Hb Kokomo (beta 74(E18) Gly>Ser)	0.22	3
Hb La Pommeraine (beta 133(H11) Val>Met)	0.22	3
Hb Brokton (beta 138(H16) Ala>Pro)	0.22	3
Hb Mito (beta 144(HC1) Lys>Glu)	0.22	3
Hb Niguarda (beta 3(NA3) Leu>Met)	<0.1	1
Total no. of cases		1330

Tab. 1

