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SHORT COMMUNICATION

HB SETIF [α94(G1)ASP→TYR] IN MALTA

Since testing of newborn infants for abnormal hemoglobins (Hbs) was reinitiated in Malta in 1989, about 20,000 cord blood samples have been studied with an isoelectrofocusing (IEF) technique (1). Among these, 2% have an abnormal Hb, ***i.e.*** 1.8% have a γ-globin variant, and 0.2% have an α-globin variant (2). β-Globin variants have been noted only sporadically. In a further survey for hemoglobinopathies in elderly members of the Maltese population, the presence of two α-globin variants that differed in amount and electrophoretic mobility, was noted (Fig. 1). One had a pI and peptide map consistent with that of Hb St. Luke's [α95(G2)Pro→Arg] which occurs at levels of around 10% of total Hb in heterozygotes from Malta (3,4). The other had a slower electrophoretic mobility and was more abundant in the red cell lysate. It was present in the proband, a healthy 88-year-old male, and in his healthy 91-year-old sister.

Methods for hematological analysis and Hb identification, quantification, and isolation are described in detail in Refs. 1 and 5. Globin chains were prepared as described by Clegg et al (6). For peptide mapping, the globin fractions were dialyzed against distilled water and 0.1% acetic acid, and digested with TPCK-trypsin for 18 hours at room temperature and pH 8.9. The tryptic peptides were then separated by reversed phase high performance liquid chromatography (HPLC) using a 1 mg sample and a Pep RPC HR 5/5 column (Pharmacia-LKB, Uppsala, Sweden). The chroma-togram was developed with a gradient of 0 to 55% acetonitrile in 0.1% aque-ous trifluoroacetic acid (TFA) over 120 minutes. The abnormal peptide was isolated, lyophilized, and analyzed on an amino acid sequencer (Porton Instruments, Tarzana, CA, USA) centered at a wavelength of 269 nm. The sequencer was set to automatically analyze up to 16 cycles and calibration, in terms of retention times, was done on 20 phenylthiohydantoin (PTH) amino acids (except cysteine) with chromatograms coming off from an on-line Hewlett Packard 3396A computing integrator (Hewlett Packard S.A., Geneva Switzerland). Alpha lactalbumin (Sigma Chemicals, St. Louis, MO, USA) was

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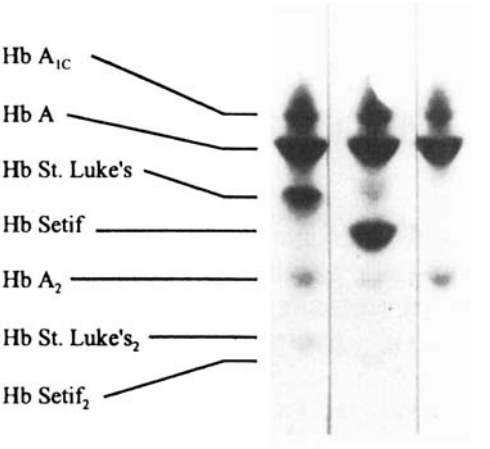

FIGURE 1

IEF of red cell lysates of a Hb St. Luke's heterozygote (lane 1), the proband with Hb Setif (lane 2), and an adult with normal Hb (lane 3).

TABLE I

Hematological and Hb Composition Data for the Proband and His Sibling

Sex-Age	RBC 10^{12}/l	PCV l/l	Hb g/dl	MCH pg	MCV fl	MCHC g/dl	X_2 %	A_2 %	X %
M-88	4.30	0.36	12.3	28.6	83.9	34.1	0.55	2.18	16.0
F-91	4.28	0.37	12.4	29.0	87.4	33.2	0.70	2.36	16.3

used as a protein standard for all sequencer-run comparisons and was dissolved in 20% acetonitrile by vortexing. The system was run on conventional Edman chemistry as used in the Porton sequencer, employing 5% phenylisothiocyanate in heptane as the coupling agent and 4% triethylamine (TEA) in water as the coupling base. A 10 µl aliquot of each peptide sample was spotted on an 8 mm Porton peptide support, prewetted in 10% acetonitrile, and dried in nitrogen prior to running the procedure. Chromatograms were developed on a reverse phase Hewlett Packard AminoQuant (C_{18})

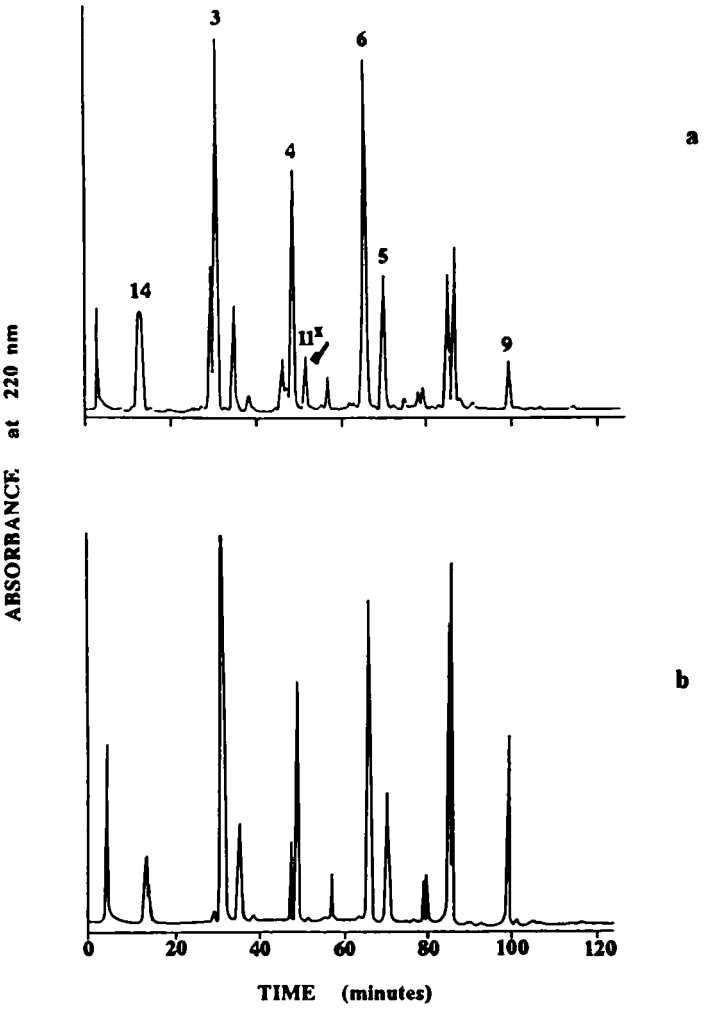

FIGURE 2

Chromatography showing the reversed phase HPLC separation of peptides obtained from the tryptic digestion of (**a**) the anomalous, and (**b**) the normal α-globin chains. One mg globin was applied on a Pep RPC HR 5/5 column (Pharmacia-LKB). The chromatogram was developed with a gradient of 0 to 55% acetonitrile in 0.1% aqueous TFA over 120 minutes.

0.21 x 20 cm narrow bore column kept at 42°C, run isocratically with a developer consisting of 100% acetonitrile as buffer B and a mix of 3.5% tetrahydrofuran (THF)/water, 29 ml 3 M sodium acetate, and 100 µl TEA, to a final pH of 3.9, as buffer A.

TABLE II

Amino Acid Sequence of the Normal and Abnormal αT-11 Peptides

Residue	93	94	95	96	97	98	99
Normal	VAL	***ASP***	PRO	VAL	ASN	PHE	LYS
Abnormal	VAL	***TYR***	PRO	VAL	ASN	PHE	LYS

The proband and his sister had normal hematology without any evidence of thalassemia or hemolytic disease (Table I). The Hb electrophoresis pattern contained an abnormal fraction which averaged 17% of the total Hb in the hemolysates from both probands. This value is nearly double that found in Hb St. Luke's heterozygotes (3,4).

Chromatography of tryptic peptides of the α-globin revealed an abnormal αT-11 peptide (Fig. 2). The amino acid sequence of the abnormal peptide (αT-11) was identical to that described for Hb Setif with an aspartic acid residue replacing a tyrosine residue at position 94 of the α-globin polypeptide (Table II) (2,3).

Although this is the first report of Hb Setif in the Central Mediterranean islands of Malta and Gozo, this variant is well-known around the Mediterranean littoral. It was first described in an Algerian male and his family (7,8), and subsequently in Cyprus, Sicily, and Spain (9-11). It has also been found among the Maltese migrant population in Australia (M.N. Cauchi, personal communication).

It is of interest that linguists and social anthropologists trace the origins of the Maltese language to the flow of migration from Maghreb to Sicily, and later southwards to Malta and Gozo (12). It seems likely that Hb Setif and its molecular haplotypes may be a suitable marker for population and gene movements in the Central Mediterranean.

Hb Setif has no pathological effect on hematological values. Indeed, in the absence of an α-thalassemia (thal), the rather high value for an α-globin variant (average 17%) indicated that it is effectively assembled in heterodimers and that the tetramers are stable *in vivo*. Although the substitution at α95(G2)Pro in St. Luke's [→Arg; (3)], Hb G-Georgia [→Leu (13)], and Hb Rampa [→Ser (14)] result in considerable dissociation of tetramers and dimers, this does not seem to be the case with substitutions at α94(G1)Asp which occur in Hb Setif (→Tyr; 16%) and Hb Sunshine Seth

[→His; 17.2% (15)] since the α94 position does not participate in α1β1 contacts. The high value reported for Hb Titusville [→Asn; 34.7%; MCV 78 fl (16)] is likely due to an associated α-thal.

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REFERENCES

1. Righetti, P.G., Gianazza, E., Bianchi-Bosisio, A., and Cossu, G., in *The Hemoglobinopathies,* edited by T.H.J. Huisman, Methods in Hematology, Vol. 15, page 47, Churchill Livingstone, Edinburgh, 1986.
2. Felice, A.E., in *Collected Papers,* edited by R.E. Micallef and S. Fiorini, page 357, University of Malta Press, University of Malta, Msida, Malta, 1992.
3. Bannister, W.H., Grech, J.L., Plese, C.F., Smith, L.L., Barton, B.P., Wilson, J.B., Reynolds, C.A., and Huisman, T.H.J., Eur. J. Biochem., 29:301, 1972.
4. Felice, A.E. and Huisman, T.H.J., Hemoglobin, 3:475, 1979.
5. Huisman, T.H.J. and Jonxis, J.H.P., *The Hemoglobinopathies Techniques of Identification,* Clinical and Biochemical Analysis, Vol. 6, page 70, Marcel Dekker, Inc., New York, 1977.
6. Clegg, J.B., Naughton, M.A., and Weatherall, D.J., J. Mol. Biol., 19:91, 1966.
7. Wajcman, H., Belkhodja, O., and Labie, D., FEBS Lett., 27:298, 1972.
8. Debray, J., Krulik, M., Mehaut, M., Benabadji, M., Trabuchet, G., Wajcman, H., Gacon, G., and Labie, D., Nouv. Rev. Fr. d'Hematol., 14:627, 1974.
9. Spivak, V.A., Aseeva, E.A., and Lutsenko, I.N., Mol. Gen. Mikrobiol. Virusol., 4:29, 1988.
10. De-Pablos, J.M., De-las-Nieves, M.A., Romero, A., Jurado, M., Garcia, A., and Moo-Penn, W.F., Sangre Barc., 35:201, 1990.
11. Schiliro, G., Russo-Mancuso, G., Dibenedetto, S.P., Samperi, P., Di Cataldo, A., Ragusa, R., and Testa, R., Hemoglobin, 15:431, 1991.

12. Brincat, J.M., in *Proceedings of the First International Colloquium on the History of the Central Mediterranean,* edited by S. Fiorini and V. Mallia Milanes, page 91, Malta University Publications, University of Malta, Msida, Malta, 1993.
13. Smith, L.L., Plese, C.F., Barton, B.P., Charache, S., Wilson, J.B., and Huisman, T.H.J., J. Biol. Chem., 247:1433, 1972.
14. De Jong, W.W.W., Bernini, L.F., and Meera Khan, P., Biochim. Biophys. Acta, 236:197, 1971.
15. Schroeder, W.A., Shelton, J.B., Shelton, J.R., and Powars, D., Hemoglobin, 3:145, 1979.
16. Schneider, R.G., Atkins, R.J., Hosty, T.S., Tomlin, G., Casey, R., Lehmann, H., Lorkin, P.A., and Nagai, K., Biochim. Biophys. Acta, 400: 365, 1975.