

Negro α -thalassaemia: genetic studies in homozygous sickle cell disease

GRAHAM R SERJEANT, KARLENE P MASON, AND BERYL E SERJEANT

From The Medical Research Council Laboratories, University of the West Indies, Kingston, Jamaica

SUMMARY Interaction with the α -thalassaemia phenotypes lowers the proportion of Hb S in the sickle cell trait and influences the mean cell volume and proportional Hb A₂ in homozygous sickle cell (SS) disease. By assigning somewhat arbitrary values to the α -thalassaemia 1 and α -thalassaemia 2 phenotypes in these conditions, it has been possible to investigate the patterns of inheritance of α -thalassaemia in black populations. The results strongly support the hypothesis that the α -thalassaemia 1 phenotype represents homozygosity for α -thalassaemia 2.

Alpha-thalassaemia in Oriental populations may be interpreted on the basis of two α -thalassaemia genotypes, α -thalassaemia 1 (deletion of a pair of linked α -chain genes, written as $--/\alpha\alpha$), and α -thalassaemia 2 (deletion of one of such a pair, written as $\alpha-/\alpha\alpha$). Homozygous α -thalassaemia 1 results in the Bart's hydrops fetalis syndrome, whereas double heterozygosity for α -thalassaemia 1 and α -thalassaemia 2 leads to haemoglobin H disease. The rarity of haemoglobin H disease and apparent absence of Bart's hydrops fetalis in Negro populations suggest that the genetics of α -thalassaemia may differ from that in Orientals. Two severities of α -thalassaemia are recognised in Negro populations, but recent observations that the α -thalassaemia 1 phenotype represents homozygosity for the α -thalassaemia 2 gene¹ raise the possibility that Negro α -thalassaemias may be explicable entirely in terms of the α -thalassaemia 2 gene.

Alpha-thalassaemia lowers the proportion of Hb S in the sickle cell trait,² the level generally differentiating the α -thalassaemia 1 and 2 phenotypes. Alpha-thalassaemia in homozygous sickle cell (SS) disease results in higher proportional Hb A₂ levels and lower mean cell volumes,³ the more extreme values occurring with the α -thalassaemia 1 phenotype. The probable α -thalassaemia phenotype may be assigned to SS patients on the basis of these values, and to their AS parents by the Hb S level. Investigating the genetics of α -thalassaemia by this method provides support for the hypothesis that the α -thalassaemia 1 phenotype in Negro Jamaican subjects represents homozygosity for α -thalassaemia 2.

Methods

Patients attended the paediatric and adult sickle cell clinics at the University Hospital of the West Indies or were part of a study based on neonatal detection of SS disease. All patients with SS disease aged over 2 years, with serum iron saturations exceeding 15%, and in whom both parents had the AS genotype with Hb S quantification, were included. Criteria for the diagnosis of SS disease are summarised elsewhere.⁴ Hb S and Hb A₂ levels

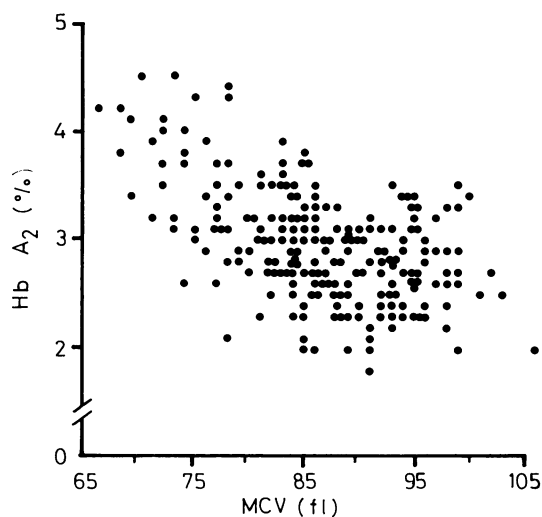


FIG 1 Relationship between mean cell volume (MCV) and proportional Hb A₂ in 215 patients with SS disease.

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were measured by elution after electrophoresis on cellulose acetate.

The distribution of MCV and proportional Hb A₂ in 215 patients (fig 1) indicates the negative relationship previously noted,³ patients with evidence of α -thalassaemia occupying the low MCV, high Hb A₂ end of this spectrum. This distribution was reanalysed according to the Hb S level in both parents. An Hb S level below 28% in an iron sufficient subject was considered indicative of the α -thalassaemia 1 phenotype, a Hb S level of 28 to 34% as indicating the α -thalassaemia 2 phenotype, and subjects with Hb S levels exceeding 34% were considered normal.

The offspring of a parent with the α -thalassaemia 1 phenotype would differ if the phenotype was attributable to heterozygous α -thalassaemia 1 or to homozygous α -thalassaemia 2 (fig 2). The exact pattern would be determined by the phenotype of the other parent and this affords three models in which to test whether the genetic data fit the heterozygous α -thalassaemia 1 or the homozygous α -thalassaemia 2 hypothesis.

Results

Values for MCV and proportional Hb A₂ in SS offspring of the putative α -thalassaemia 1 phenotype

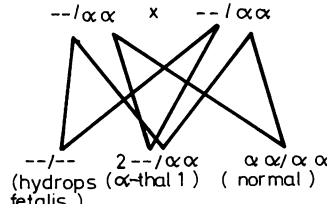
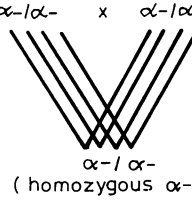
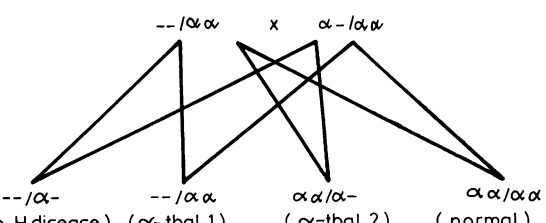
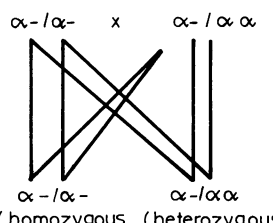
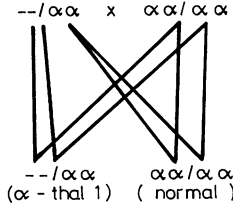
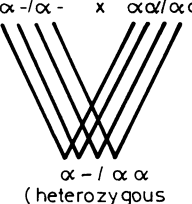
Phenotype of second parent	Genetic basis of α -thalassaemia 1 phenotype	
	(a) Heterozygous α -thalassaemia 1	(b) Homozygous α -thalassaemia 2
α -thalassaemia 1 (model 1)	 <p>--/αα x --/αα</p> <p>--/-- (hydrops fetalis) --/αα (α-thal 1) αα/αα (normal)</p>	 <p>α-/α- x α-/α-</p> <p>α-/α- (homozygous α-thal 2)</p>
α -thalassaemia 2 (model 2)	 <p>--/αα x α-/αα</p> <p>--/α- (Hb H disease) --/αα (α-thal 1) αα/α- (α-thal 2) αα/αα (normal)</p>	 <p>α-/α- x α-/αα</p> <p>α-/α- (homozygous α-thal 2) α-/αα (heterozygous α-thal 2)</p>
Normal (model 3)	 <p>--/αα x αα/αα</p> <p>--/αα (α-thal 1) αα/αα (normal)</p>	 <p>α-/α- x αα/αα</p> <p>α-/αα (heterozygous α-thal 2)</p>

FIG 2 Expected offspring of a parent with the α -thalassaemia phenotype attributable to (a) heterozygous α -thalassaemia 1 and (b) homozygous α -thalassaemia 2. Three models are available according to phenotype of second parent.

are shown in fig 3a. The first model in fig 2 could not be tested since no offspring were born to matings where both parents had the α -thalassaemia 1 phenotype. Offspring of the second model, depicted as open circles in fig 3a, fell into two groups compatible with homozygous and heterozygous α -thalassaemia 2. Offspring of the third model,

depicted as closed circles in fig 3a, represent a homogeneous group compatible with the heterozygous α -thalassaemia 2 offspring of the second model. These results are therefore only compatible with the hypothesis that the α -thalassaemia 1 phenotype represents homozygous α -thalassaemia 2.

The dotted lines in fig 3a divide the SS data into

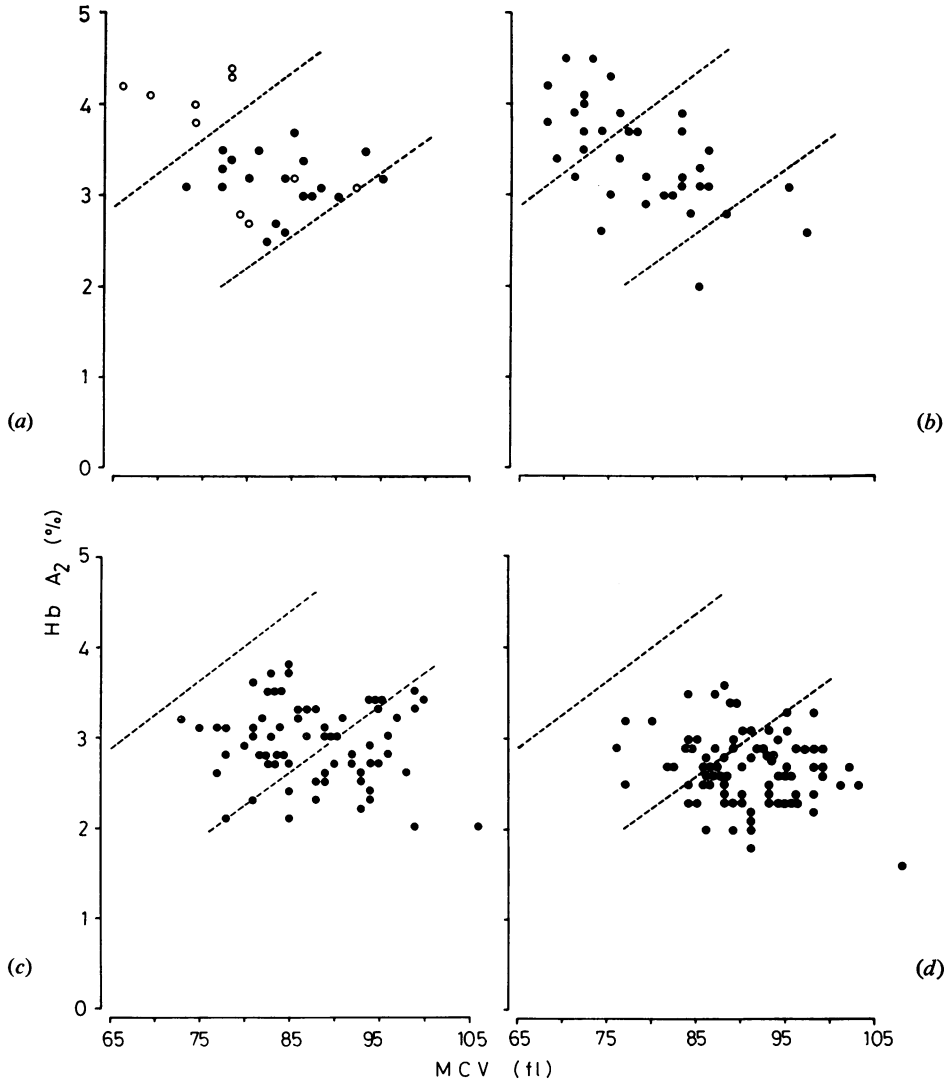


FIG 3 Relationship between MCV and proportional Hb A₂ in SS offspring of AS subjects with α -thalassaemia or normal phenotypes. (a) α -thalassaemia 1/ α -thalassaemia 2 matings (O), n = 10; α -thalassaemia 1/normal matings (●), n = 19; (b) α -thalassaemia 2/ α -thalassaemia 2, n = 35; (c) α -thalassaemia 2/normal, n = 68; (d) normal/normal, n = 83.

Dotted lines divide presumed α -thalassaemia 2 homozygotes, α -thalassaemia 2 heterozygotes, and normal populations (see text).

probable α -thalassaemia phenotypes, the upper line separating presumed α -thalassaemia 2 homozygotes from obligate α -thalassaemia 2 heterozygotes, and the lower line parallel to the upper but enclosing 95% of the obligate α -thalassaemia 2 heterozygotes.

Data on offspring of α -thalassaemia 2/ α -thalassaemia 2, α -thalassaemia 2/normal, and normal/normal matings are shown in fig 3b, c, and d, respectively. Offspring of α -thalassaemia 2/ α -thalassaemia 2 matings would be expected to be one quarter α -thalassaemia 2 homozygotes (that is, α -thalassaemia 1 phenotypes), one half α -thalassaemia 2 heterozygotes, and one quarter normal, the observed distribution being 13:19:4. However, many families were ascertained by the investigation of SS patients apparently homozygous for α -thalassaemia 2 and this selection will have biased the findings. Offspring of α -thalassaemia 2/normal matings should be half α -thalassaemia 2 heterozygotes (between the two lines) and half normal (below the lower line), the observed division being 39:29. Offspring of normal/normal matings, as expected, fell predominantly below the lower line. Neither of the latter two matings could produce α -thalassaemia 2 homozygotes and no offspring occur about the upper line.

Discussion

Evidence that α -thalassaemia in SS disease results in low MCV values and a rise of proportional Hb A₂³ suggested that these indices might be useful in differentiating subgroups of SS disease resulting from the interaction with different α -thalassaemia phenotypes. In practice, arbitrary lines derived from presumed α -thalassaemia 2 homozygotes and obligate α -thalassaemia 2 heterozygotes fitted the different models reasonably successfully. The upper line indicated α -thalassaemia 2 homozygotes only in matings where it was compatible with parental genotype. The lower line bisected reasonably accurately (57% : 43%) offspring of α -thalassaemia 2/normal matings, but 27% of offspring of normal/

normal matings fell above this line. However, in view of the overlap of haematological indices and of globin chain ratios between α -thalassaemia 2 heterozygotes and normal subjects, the lack of a clearer differentiation is not surprising.

The assignment of different Hb S levels to α -thalassaemia phenotypes in the sickle cell trait was, to some extent, arbitrary. Hb S levels below 28% were selected for homozygous α -thalassaemia 2 because levels of 25.2 and 26.7% occurred in subjects with this genotype confirmed by DNA restriction mapping¹ and because the distribution of Hb S levels in parents indicated a small but separate subpopulation with Hb S levels below 28%. The differentiation of α -thalassaemia 2 heterozygotes from normal subjects by a level of 34% was more arbitrary. The distribution of Hb S levels showed a small antimode at 34% and Hb S levels between 31 to 33% occurred in three subjects shown by restriction mapping to be α -thalassaemia 2 heterozygotes.¹

The acceptably close fit of the data to the expected models suggests the validity of these assumptions and supports the hypothesis that the α -thalassaemia 1 phenotype observed in Negro Jamaican subjects represents homozygosity for the α -thalassaemia 2 gene.

References

- ¹ Higgs DR, Pressley L, Old JM, Hunt DM, Clegg JB, Weatherall DJ, Serjeant GR. Negro α -thalassaemia is caused by deletion of a single α -globin gene. *Lancet* 1979;ii:272-6.
- ² Steinberg MH, Adam JC, Dreiling BJ. Alpha thalassaemia in adults with sickle-cell trait. *Br J Haematol* 1975;30:31-7.
- ³ Sewell A, Millard DP, Serjeant GR. The interaction of alpha thalassaemia with SS disease. *Prog Clin Biol Res* 1978;2:93-107.
- ⁴ Serjeant GR. *The clinical features of sickle cell disease*. Amsterdam: North Holland Publishing Co, 1974:59-62.

Requests for reprints to Dr G R Serjeant, MRC Laboratories (Jamaica), University of the West Indies, Mona, Kingston 7, Jamaica, WI.