conclude that the Turner-like features associated with 18p— may be determined by monosomy for 18p11.

Abnormalities of the short arm of chromosome 18 show distinctive phenotypes depending on the number of copies of this area present (table). Monosomy 18p has a Turner-like phenotype with moderate mental retardation. Interestingly, trisomy 18p shows little phenotypic effect and mental development has ranged from normal² to mildly delayed.⁷ Tetrasomy 18p has severe phenotypic features including moderate to severe mental retardation, hypotonia, and multiple musculoskeletal anomalies.

Abnormalities of 18q have greater phenotypic effects. Complete monosomy of 18q has not been described, but a fairly consistent phenotype (table), which includes severe mental retardation, has been reported in partial 18q—. Patients who are trisomic for 18q and disomic for 18p have a typical trisomy 18 phenotype, indicating that only trisomy 18q is required for full expression of this phenotype.⁵

Formation of isodicentric chromosomes is poorly understood. If formation occurs during mitotic or first meiotic division, it would result in a derivative chromosome with non-identical arms and centromeres. However, formation during second meiotic division would result in identical arms and centromeres. We are not aware of chromosome 18 polymorphism or polymorphic gene markers that would allow us to choose among these options. The majority of reported dicentric chromosomes have had only one active centromere.⁸ We currently do not understand the centromere inactivation process. Possibly, suppression of one centromere results in a more stable structure that is better able to undergo cell division.

Our report of an isopseudodicentric(18) associated with a trisomy 18 phenotype adds information to the correlation of phenotype and genotype in chromosome 18 abnormalities. Considering the vast phenotypic differences in normal subjects, it is not surprising that phenotypic variation exists in those with similar chromosome abnormalities. It is more remarkable that sufficient similarity exists among these patients to allow phenotype-genotype correlation.

References

- 1 de Grouchy J. The 18p—, 18q—, and 18r syndromes. *Birth Defects* 1969;**5**:74–87.
- 2 Taylor KM, Wolfinger HL, Brown MG, Chadwick OL. Origin of a small metacentric chromosome: familial and cytogenetic evidence. *Clin Genet* 1975;**8**:364–9.
- 3 Nielsen KB, Dyggve H, Friedrich V, Hobolth N, Lyngbye T, Mikkelsen M. Small metacentric nonsatellited extra chromosome: report of five mentally retarded individuals and review of literature. *Hum Genet* 1978;**44**:59–69.
- 4 de Grouchy J, Turleau CT. *Clinical atlas of human chromosomes*. New York: Wiley, 1977:170–6.
- 5 Hecht F, Bryant J, Arakaki D, Kaplan E, Gentile G. Trisomy-18 syndrome due to de-novo translocation. *Lancet* 1963;**i**:114.
- 6 Bass HN, Sparkes RS, Miller AA. Features of trisomy 18 and 18p— syndromes in an infant with 46,XY, i(18q). *Clin Genet* 1979;**16**:163–8.
- 7 Jacobsen P, Mikkelsen M. Chromosome 18 abnormalities in a family with a translocation 5(18p—, 21p—). *J Ment Defic Res* 1969;**12**:144–61.
- 8 Madan K, Vlasveld L, Barth PG. Ring-18 and isopseudodicentric-18 in the same child: a hypothesis to account for common origin. *Ann Genet (Paris)* 1981;**24**:12–6.

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A genetic combination of silent β -thalassaemia, high Hb A₂ β -thalassaemia, and single α globin gene deletion causing mild thalassaemia intermedia

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SUMMARY This paper reports a Sardinian patient, who was a compound heterozygote for silent β -thalassaemia and high Hb A₂ β °-

thalassaemia with the clinical phenotype of mild thalassaemia intermedia; α globin gene mapping showed a single α globin gene deletion. The reduced α globin chain output resulted in more balanced globin chain synthesis, which in turn accounted for the mild clinical phenotype.

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Compound heterozygotes for 'silent' β -thalassaemia and high Hb A₂ β -thalassaemia, either of the β^0 or β^+ type, show a variable clinical phenotype ranging from mild thalassaemia intermedia to an attenuate form of thalassaemia major characterised by later onset (around 3 to 5 years) of transfusion dependence.¹⁻³ The molecular basis of this heterogeneity has not yet been elucidated.

We report a Sardinian compound heterozygote for silent β -thalassaemia and high Hb A₂ β^0 -thalassaemia with the clinical phenotype of mild thalassaemia intermedia in which restriction endonuclease analysis showed a single α globin structural gene deletion.

In this case, the association of α -thalassaemia reduced the globin chain imbalance and thus accounted for the mild clinical phenotype.

Methods

Haematological data were obtained with a Coulter Counter model S. Haemoglobin A₂ levels were determined by microchromatography⁴ and Hb F levels by alkali denaturation.⁵ Globin chain synthesis studies were performed according to Kan *et al.*⁶ Globin chain separation was carried out by isoelectric focusing⁷ and by electrophoresis on acid urea Triton acrylamide gel.⁸ Other values were obtained by standard methods.

DNA restriction enzyme analysis was performed according to Goossens and Kan.⁹ *Bam* HI and *Bgl* II digests were hybridised with nick-translated α and ζ globin gene probes.

The β globin gene polymorphisms were studied by the method of Antonarakis *et al.*¹⁰

Case report

The proband (II.1, fig 1), the first child of a non-consanguineous mating, was referred to our service at 11 years of age because of pallor and slight jaundice which had been noted from the first year of life.

His weight was 26 kg (<3rd centile) and his height 130 cm (<3rd centile). Physical examination showed moderate pallor and jaundice, slight enlargement of the liver (lower margin 3 cm below the costal margin) and spleen (lower tip 4 cm below

FIG 1 Family pedigree.

the costal margin), and mild thalassaemia-like skeletal changes. X-ray examination showed dilation of the diploic space and porous rarefaction of long bones.

Pertinent haematological data are summarised in table 1. He had a moderate microcytic anaemia with raised Hb A₂ (4.82%) and Hb F (8.75%) levels. Blood film showed typical thalassaemia-like red blood cell abnormalities and rare nucleated red blood cells (three per 100 white blood cells). Unconjugated bilirubin level was 0.70 g/l, transferrin saturation 26%, and serum ferritin 117 γ %,

TABLE 2 Haplotypes of polymorphic restriction sites.

	<i>Hinc</i> II 5' ϵ	<i>Hind</i> III G γ	A γ	<i>Hinc</i> II 3' Ψ β_1	<i>Ava</i> II β	<i>Bam</i> HI 3' β
Proband	-/-	+/+	+/+	-+/+	+/+	+/+
Father	+/+	-/+	-/+	-/-	+/+	+/+
Mother	-/-	+/+	+/+	-+/+	+/+	+/+

Underlined symbols indicate the haplotype of the β -thalassaemia silent chromosome.

TABLE 1 Haematological data and globin chain synthesis analysis.

	Age (yr)	RBC ($\times 10^{12}/l$)	Hb (g/dl)	MCV (fl)	MCH (pg)	Reticulocytes (%)	Hb A ₂ (%)	Hb F (%)	α/β ratio
II.1	11	4.9	9.4	62	19.2	4	4.82	8.7	2.47*
II.2	2.6	5.8	10.8	58	18.6	2.5	4.39	11.0	2.02*
I.1	38	5.5	15.8	85	28.9	1	2.99	0.5	1.92
I.2	35	5.5	13.8	78	25.0	1.2	4.64	0.6	0.76

* = $\alpha/\beta+\gamma$

The α /non- α globin chain synthesis ratio was 2.47. The G γ chains were 55% of the total γ chains. Electrophoresis of globin chains in acid urea Triton acrylamide excluded the presence of Hb Knossos which is associated with an increased α / β ratio within the range of the β -thalassaemia silent carrier state and, thus, when interacting with a high Hb A₂ β -thalassaemia gene, produces thalassaemia intermedia.¹¹

The bone marrow was markedly cellular with a striking erythroid hyperplasia (myeloid/erythroid ratio 1:5).

Follow up of this patient over 3 years showed fluctuating Hb levels which, however, never fell below 8 g/dl.

Restriction endonuclease analysis with *Bgl* II and *Bam* HI and hybridisation with α and ζ globin specific probes showed a pattern indicative of the single α globin gene deletion ($-\alpha/\alpha\alpha$) produced by the rightward deletion crossover mechanism (fig 2).

Analysis of the restriction enzyme site polymorphisms within and around the β -like gene cluster is shown in table 2. The high Hb A₂ β -thalassaemia allele seems to be linked with the ($-++-++$) haplotype, while the silent β -thalassaemia mutation appears to be associated with the

($-++-++$ $+-$) type. In Sardinians, the ($-++-++$ $++$) haplotype is the most frequent among the different haplotypes linked to the commonest and perhaps unique β^0 -thalassaemia mutation (nonsense β^{39}) in this population¹² (Pirastu and Kan, unpublished data). Thus, the high Hb A₂ β -thalassaemia mutation in our proband is very likely the common Sardinian β^0 amber termination mutant.

FAMILY EXAMINATION

The sister, aged 2½ years, showed a clinical and haematological picture and an α globin genotype ($-\alpha/\alpha\alpha$) similar to that of the proband (fig 2). However, her Hb levels were consistently higher and the enlargement of the spleen and liver was less marked. This milder clinical expression may be because of her younger age, as in large series of patients with the silent β -thalassaemia/high Hb A₂ β -thalassaemia combination the clinical picture was seen to deteriorate with advancing age.²

The mother, of Sardinian extraction, showed the haematological phenotype typical of the β -thalassaemia carrier state, with high Hb A₂ (4.64%) and traces of Hb F (0.6%). Restriction endonuclease analysis revealed the deletion of one α globin structural gene in each chromosome ($-\alpha/-\alpha$), which accounts for the reduced α / β globin chain synthesis ratio and the absence of microcytosis.^{13 14}

The father, of southern Italian origin, showed a normal haematological phenotype and a full complement of four α structural genes ($\alpha\alpha/\alpha\alpha$). However, the α / β globin chain synthesis ratio was unbalanced (1.92%), indicating a heterozygous state for the silent β -thalassaemia mutation.^{3 15}

Discussion

This study describes a patient with the clinical phenotype of mild thalassaemia intermedia produced by a complex combination of α - and β -thalassaemia genes. Genetic evidence indicates that he inherited a silent β -thalassaemia gene from his father and a high Hb A₂ β -thalassaemia gene, very likely of the non- β chain producing variety, from his mother. Restriction endonuclease mapping showed the deletion of one of the four α globin structural genes which was transmitted from his mother.

Previous studies have shown that patients with the combination of β^0 - or β^{+} -thalassaemia genes and silent β -thalassaemia may have a remarkably heterogeneous clinical picture, the severity of which varies from that of thalassaemia intermedia to an, albeit attenuate, transfusion dependent thalassaemia major.¹⁻³

The proband described here is an example of the less severe clinical expression associated with the

FIG 2 Autoradiogram of *Bam* HI digested DNA hybridised with an α globin specific probe. 1 proband, 2 sister, 3 father, 4 mother.

aforementioned genotype. Thus, it is reasonable to assume that these milder manifestations depend on a lesser globin chain imbalance which, in turn, is caused by the association of α -thalassaemia. This association has already been seen to ameliorate the clinical and haematological expression of β^+ - and β^0 -thalassaemia both in the homozygous and heterozygous states.¹⁻³

[In families such as this, if antenatal diagnosis is requested it should be done by DNA analysis of amniotic fluid cells. In addition to the higher risk to the fetus, in these cases globin chain synthesis analysis of fetal blood could produce misleading results.

References

- ¹ Aksoy M, Dincol G, Erdem S. Different types of beta thalassaemia intermedia. A genetic study in 20 patients. *Acta Haematol* 1978;**59**:178-89.
- ² Kattamis C, Mataxatou-Mavromati A, Wood WG, Nash JR, Weatherall DJ. The heterogeneity of normal Hb A₂ β -thalassaemia in Greece. *Br J Haematol* 1979; **42**:109-23.
- ³ Weatherall DJ, Clegg JB. *The thalassaemia syndromes*. 3rd ed. Oxford: Blackwell Scientific Publications, 1981: 260.
- ⁴ Huisman THJ, Schroeder W, Brodie AN, Mayson SM, Jakway J. Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A₂. *J Lab Clin Med* 1975;**86**:700-2.
- ⁵ Pembrey ME, McWade P, Weatherall DJ. Reliable routine estimation of small amounts of foetal haemoglobin by alkali denaturation. *J Clin Pathol* 1972;**25**: 738-40.
- ⁶ Kan YW, Schwartz E, Nathan DG. Globin chain synthesis in alpha thalassemia syndromes. *J Clin Invest* 1968;**47**:2515-22.
- ⁷ Valkonen K, Giannazza E, Righetti PG. Human globin chain separation by isoelectric focusing in ultrathin polyacrylamide gels. *Clin Chim Acta* 1980;**107**:223-9.
- ⁸ Alter BP, Goff SC, Efremov GD, Gravely ME, Huisman THJ. Globin chain electrophoresis: a new approach. *Br J Haematol* 1980;**44**:527-34.
- ⁹ Goossens M, Kan YW. DNA analysis in the diagnosis of hemoglobin disorders. In: *Methods in enzymology*. New York: Academic Press, 1981:805.
- ¹⁰ Antonarakis SE, Boehm CD, Giardina PJV, Kazarian HH Jr. Nonrandom association of polymorphic restriction sites in the β -globin gene cluster. *Proc Natl Acad Sci USA* 1982;**79**:137-41.
- ¹¹ Fessas P, Loukopoulou D, Loutradi-Anagnostou A, Komis G. 'Silent' β -thalassaemia caused by a 'silent' β -chain mutant: the pathogenesis of a syndrome of thalassaemia intermedia. *Br J Haematol* 1982;**51**:577-83.
- ¹² Trecartin RF, Liebhaber SA, Chang JC, *et al.* β^0 -thalassaemia in Sardinia is caused by a nonsense mutation. *J Clin Invest* 1981;**68**:1012-7.
- ¹³ Kanavakis E, Wainscoat JS, Wood WG, *et al.* The interaction of α -thalassaemia with heterozygous β -thalassaemia. *Br J Haematol* 1982;**52**:465-73.
- ¹⁴ Melis MA, Pirastu M, Galanello R, Furbetta M, Tuveri T, Cao A. Phenotypic effect of heterozygous α and β^0 -thalassaemia interaction. *Blood* 1983;**62**:226-9.
- ¹⁵ Schwartz E. The silent carrier of beta thalassemia. *N Engl J Med* 1969;**281**:1327-33.

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