Vol. 91, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1979 Pages 548-553

RESTRICTION ENZYME ANALYSIS OF THE β-GLOBIN GENE IN DNA FROM β°-THALASSAEMIC SUBJECTS FROM FERRARA

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Received October 2, 1979

SUMMARY

The map of restriction sites including and surrounding the δ- and β-globin genes has been established for three Ferrara β°-thalassaemic subjects. The fragments obtained using nine restriction enzymes do not show any differences from normal DNA. Among others, restriction enzymes giving short fragments at the 5' and 3' ends of the β-globin structural gene have been employed. The results obtained for the thalassaemic DNA are identical to those for control DNA, thus excluding the presence of extensive deletions in or adjacent to the coding regions of the β-globin gene in Ferrara β°-thalassaemia.

Thalassaemias are a group of inherited anaemias in which the synthesis of one of the globin chains of haemoglobin is reduced or absent.

Ferrara thalassaemia is a β°-thalassaemia: HbA is not detectable in the red blood cells of homozygous subjects (1). β-Globin synthesis does not occur in thalassaemic cells incubated with labelled amino acids (2), and it is impossible to detect radioactive β-globin peptides from thalassaemic reticulocytes as products of incomplete translation of β-globin messenger RNA (3).

There is evidence indicating that in Ferrara β°-thalassaemia β-globin mRNA is present. Thalassaemic reticulocyte ribosomes synthesise β-globin when incubated in the presence of ribosomal supernatant from normal or HbS reticulocytes (4), suggesting the presence of a functional but untranslated

0006-291X/79/220548-06$01.00/0

β-globin mRNA. Hybridisation of RNA extracted from Ferrara β°-thalassaemic red blood cells has demonstrated the presence of mRNA for β-globin (5). Ramirez et al. (6), in other hybridisation experiments, have obtained contrary data interpreted as indicating abnormal β-globin mRNA in Ferrara β°-thalassaemia.

The development of the Southern method (7) for the identification of gene-specific DNA sequences after restriction endonuclease digestion and gel electrophoresis has allowed detection, sizing and mapping of globin DNA sequences from mouse and rabbit (8-10). These mthods have recently been applied to define the "restriction map" of the human DNA fragments containing the γ-, δ- and β-globin gene family (11-13). The map obtained for the δ- and β-globin gene locus, with the restriction endonuclease cleavage sites used in this study indicated, is shown in Fig. 1.

We have used the same technique with Ferrara β°-thalassaemic DNA to verify the presence of the δ- and β-globin genes and to attempt to identify an abnormality characteristic of this type of thalassaemia.

METHODS

Cellular DNA was isolated from spleens removed from three Ferrara thalassaemic subjects and from a normal placenta. All three thalassaemic subjects synthesised no β-globin in whole cells incubated with labelled amino acids. The three cases

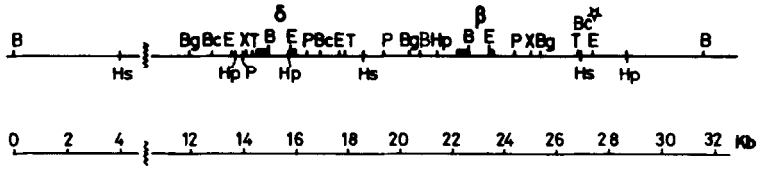

FIGURE 1: Map of the δ- and β-globin gene locus (12). The cleavage sites for the restriction enzymes EcoRI (E), BglII (Bg), PstI (P), XbaI (X), TaqYI (T), BamHI (Ba), BclI (Bc), HsuI (H) and HpaI (Hp) nearest to the coding sequence, and thus generating fragments hybridising to the plasmid probe used, are shown. The starred BclI site has not been accurately located.

analysed were also typical Ferrara β°-thalassaemics 1) by the presence of hybridisable β-globin RNA sequences (5); 2) by the ability of their ribosomes to synthesise β-globin when incubated in the presence of normal, RNase-treated supernatant (4), and by the appearance in their circulating reticulocytes of β-globin synthesis after transfusion with normal packed red blood cells, a phenomenon typical of Ferrara β°-thalassaemia and not found in β°-thalassaemias from other regions (14).

The DNA preparations, restriction enzyme digestions, electrophoresis, Southern transfer and filter hybridisation were as previously described (12). The β-globin probe used was the plasmid pHβGl (15), which contains β-globin cDNA inserted in plasmid pCR1, and this recombinant was handled under Category II conditions in the London laboratory as advised by the U.K. Genetic Manipulation Advisory Group.

RESULTS

Fig. 2 shows the autoradiography pattern of the fragments obtained after digestion of normal and Ferrara β°-thalassaemic DNA with the restriction enzymes EcoRI, BglII, PstI and XbaI respectively. Because of the homology between δ- and β-globin genes, both hybridise with the β-globin plasmid DNA probe (12). No differences are evident between control and thalassaemic DNA. Five other restriction enzymes have been used; BamHI, TaqYI, BclI,

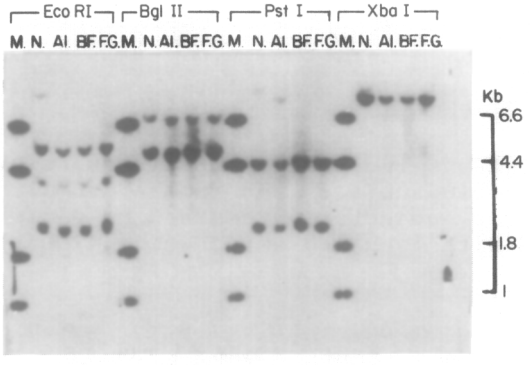

FIGURE 2: Autoradiography pattern of δ- and β-globin gene fragments in digests of normal and Ferrara β°-thalassaemia homozygotes (A.I., B.F., F.G.) were digested to completion with EcoRI, BglII, PstI and XbaI. 20 μg of each DNA digest was applied and electrophoresed on an 0.8% agarose gel. Hybridisation marker (M) was 20 pg human plasmid pHγGl DNA, digested with BglI. After denaturation the DNA was transferred by blotting to a nitrocellulose filter (7). 32P-labelled pHβGl DNA was used to detect DNA fragments containing δ- and β-globin gene sequences (12). The faint band seen at approximately 13 kb in normal DNA digested with EcoRI or PstI is a plasmid contaminant.

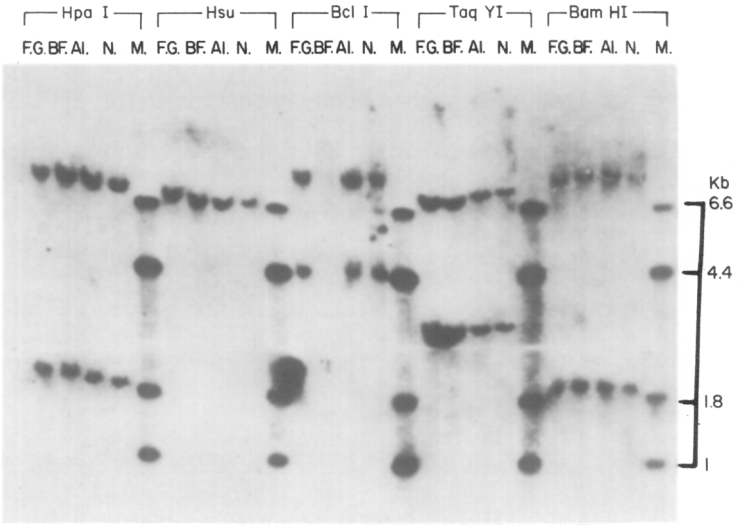

FIGURE 3: δ- and β-globin gene fragments of control and Ferrara β°-thalassaemia DNA after digestion with BamHI, TaqYI, BclI, HsuI and HpaI. The digestion of the DNA sample from patient B.F. with BclI did not go to completion.

HsuI and HpaI (Fig. 3). In every case no differences between normal and Ferrara β°-thalassaemic DNA are detectable.

DISCUSSION

The structure and organisation of the δ- and β-globin genes have been studied in Ferrara β°-thalassaemia using the procedures of Flavell et al. (12), which allow the mapping of restriction sites within these two genes and in surrounding DNA sequences.

The data obtained show that the sizes of the various restriction fragments in the normal DNA used as control are the same as those previously reported (12). No differences between normal and Ferrara β°-thalassaemic DNA have been detected. These findings demonstrate a normal arrangement for the δ- and β-globin genes in this type of thalassaemia.

It has been suggested that the β-globin mRNA sequences from Ferrara β°-thalassaemia reticulocytes do not completely saturate

full length β-globin cDNA. If this is true, one possibility is that only the 5'-sequence of the mRNA is present, owing to a deletion of the 3'-portion of the gene, as has been demonstrated for several cases of β°-thalassaemia from places other than Ferrara (16-18). There are, of course, other possible mechanisms which would give a partial mRNA transcript, such as premature release of RNA polymerase. The findings reported here exclude detectable deletions in the structural genes, the intervening sequences and the flanking regions of the δ- and β-globin genes in Ferrara β°-thalassaemia.

In addition, BamHI and EcoRI, which recognise the codons for amino acids 98-100 and 121-122 respectively in δ- and β-globin genes, generate the expected fragments, demonstrating structural normality for these regions of the coding sequence (19). In human DNA, BamHI digestion gives a 1.8 kb fragment containing the 5'-end of the β-globin gene, while a double digest with EcoRI and PstI yields a 1 kb fragment including the 3'-end of the β-globin gene. Because of the small size of these DNA fragments, even short deletions of approximately 100 base pairs would cause an altered electrophoretic mobility and therefore be detected. The identical patterns obtained in normal and in Ferrara β°-thalassaemia DNAs therefore exclude deletions of this size or larger in or adjacent to the thalassaemic β-globin gene (double digest data not shown).

In conclusion, the data presented provides evidence for the lack of demonstrable differences between normal and Ferrara β°-thalassaemia DNA with nine restriction enzymes. These findings are those expected from previous data, which indicate the presence of β-globin mRNA in Ferrara β°-thalassaemia both by translation (4) and by cDNA hybridisation (5). It also shows the absence of DNA polymorphisms in the Ferrara β°-thalassaemia gene for the sequences recognised by the restriction enzymes used, indicating that at least for these enzymes

antenatal diagnosis using the approach pioneered by Kan and Dozy (20) for sickle cell disease will not be applicable.

ACKNOWLEDGEMENTS

This work was supported in part by the Italian Consiglio Nazionale dell Ricerche, by the Italian della Pubblica Istruzione and by Cassa di Risparmio di Ferrara, and by grants to R.W. from the U.K. M.R.C. and the U.S. National Institutes of Health (1R01AM20215-01A1). An EMBO short term fellowship allowed Dr. L. del Senno to visit the Department of Biochemistry, St. Mary's Hospital Medical School.

REFERENCES

1. Bargellesi, A., Pontremoli, S. & Conconi, F. (1967) Eur.J.Biochem. 1, 73-79.
2. Conconi, F., Bargellesi, A., Pontremoli, S., Vigi, V., Volpato, S. & Gaburro, D. (1968) Nature 217, 259-260.
3. Dreyfus, J.C., Labie, D., Vibert, M. & Conconi, F. (1971) Eur.J. Biochem. 27, 291-296.
4. Conconi, F., Rowley, P.T., Del Senno, L., Pontremoli, S. & Volpato, S. (1972) Nature 238, 83-87.
5. Ottolenghi, S., Comi, P., Giglioni, B., Williamson, R., Vullo, C., Del Senno, L. & Conconi, F. (1977) Nature 266, 231-234.
6. Ramirez, F., O'Donnell, J.V., Marks, P.A., Bank, A., Musemeci, S., Schiliro, G., Pizzarelli, G., Russo, G., Luppis, B. & Gambino, R. (1976) Nature 263, 471-475.
7. Southern, E.M. (1975) J.Mol.Biol. 98, 503-517.
8. Jeffreys, A.J. & Flavell, R.A. (1977) Cell 12, 429-439.
9. Jeffreys, A.J. & Flavell, R.A. (1977) Cell 12, 1097-1108.
10. Tilghman, S.M., Tiemeier, D.C., Polsky, F., Edgell, M.H., Seidman, J.G., Leder, A., Enquist, L.W., Norman, B. & Leder, P. (1977) Proc.Natl.Acad.Sci.U.S. 74, 4406-4410.
11. Little, P.R.F., Flavell, R.A., Kooter, J.M., Annison, G. & Williamson, R. (1979) Nature 278, 227-231.
12. Flavell, R.A., Kooter, J.M., De Boer, E., Little, P.F.R. & Williamson, R. (1978) Cell 15, 25-41.
13. Mears, J.G., Ramirez, F., Leibowitz, D. & Bank, A. (1978) Cell 15, 15-23.
14. Conconi, F., Del Senno, L., Ferrarese, P., Menini, C., Borgatti, L., Vullo, C. & Labie, D. (1975) Nature 254, 256-259.
15. Little, P.F.R., Curtis, P., Coutelle, Ch., Van Den Berg, J., Dalgleish, R., Malcolm, S., Courtney, M., Westaway, D. & Williamson, R. (1978) Nature 273, 640-643.
16. Old, J.M., Proudfoot, N.J., Wood, W.G., Longley, J.I., Clegg, J.B. & Weatherall, D.J. (1978) Cell 15, 15-23.
17. Orkin, S.H., Old, J.M., Weatherall, D.J. & Nathan, D.G. (1979) Proc.Natl.Acad.Sci.U.S. 76, 2400-2404.
18. Flavell, R.A., Bernards, R., Kooter, J.M., De Boer, E., Little, P.F.R., Annison, G. & Williamson, R. (1979) Nucleic Acid Res. 6, 2749-2760.
19. Marrota, C.A., Wilson, J.T., Forget, B.G. & Weissman, S.M. (1977) J.Biol.Chem. 252, 5040-5053.
20. Kan, Y.W. & Dozy, A.M. (1978) Lancet ii, 190-192.