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# GENE MAPPING OF HUMAN BILIRUBIN UDP-GLUCURONOSYL TRANSFERASE ON 1q21-q23 BY A CELL SORTER AND *IN SITU* HYBRIDIZATION

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*Summary* The human liver bilirubin UDP-glucuronosyl transferase (bilirubin UDPGT) [EC 2.4.1.17] is responsible for the enzyme deficiency in Crigler-Najjar syndrome and/or Gilbert's syndrome. The UDPGT, former shows severe jaundice resulted from a complete absence of bilirubin while the latter has a mild manifestation due to a reduction of the enzyme activity. The gene locus of bilirubin UDPGT was mapped to chromosome 1 by spot-blot hybridization using a cell-sorter, and its regional locus was assigned to 1q21-q23 by high resolution *in situ* hybridization.

***Key Words*** spot-blot hybridization, *in situ* hybridization, UDP-glucuronosyl transferase, cell-sorter, gene mapping

## INTRODUCTION

Recently the specific chromosome regions of many human genes and hereditary diseases have been determined and the results of such gene mapping are rapidly being accumulated at the every Workshop on Human Gene Mapping. Deficiency of bilirubin UDPGT causes Crigler-Najjar syndrome and/or Gilbert's syndrome. As for Crigler-Najjar syndrome-type 1, it is a complete deficiency of bilirubin UDPGT and shows an autosomal recessive trait. In this disorder, glucuronization in the liver is not achieved, and thus bilirubin is not conjugated, which results in severe jaundice and neonatal intoxication. Infant thus affected die in the neonatal period due to kernikterus. In Crigler-Najjar syndrome-type 2, which may be the

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same as Gilbert's syndrome, is characterized by a mild manifestation of jaundice and is thought an autosomal trait inheritance (Black and Billing, 1969).

In 1987, Jackson *et al.* cloned the cDNA (HLUG 25) of bilirubin UDPGT gene and sequenced the full length of its cDNA. Using a 5′ *Eco*RI fragment of this cDNA, its locus was determined to be on chromosome 1 using sorted chromosomes, and more specifically to be in the 1q21-q23 region by high resolution *in situ* hybridization.

## MATERIALS AND METHODS

*1) DNA probe.* A cDNA clone (HLUG 25) encoding the complete sequence bilirubin UDPGTwas isolated from a λgt11 human liver cDNA library (Jackson of a *et al.*, 1987). The sequence of HLUG 25 cDNA was determined to be 2,104 base-pair long, including a poly(A) tail a long open reading frame. We used a 1.2 kb *Eco*RI fragment of HLUG 25 as a probe for gene mapping.

*2) Chromosome sorting and spot blot hybridization.* Preparation of metaphase chromosome suspension was according to Lebo *et al.* (1985) and Minoshima *et al.* (1990) with several modifications. The spontaneously transformed human B-lymphoblastoid cell lines GM00130B (46, XY) was obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). Metaphase chromosomes stained with Hoechst 33258 were excited with 319 nm light (200mW) with an argon ion laser. Chromosomes were analyzed and sorted uing a fluorescence-activated FACS440 cell sorter (Becton-Dickinson). Eighty thousand of each type were sorted directly onto nitrocellulose membrane filter disks (13 mm diameter, Schleicher & Schuell). The chromosomal DNA was denatured and baked at 80°C for 2 hr in a vacuum. The filters were prehybridized for 12 hr at 42°C, and then hybridized with $^{32}$P-labeled probe for 24 hr at 42°C. After hybridization, the filters were washed, and autoradiographed with an intensifier screen on Fuji RX X-ray film at −70°C for 12–24 hr.

*3) High resolution in situ hybridization.* Chromosome sample was prepared according to the method of Dutrillaux and Vigas-Pequignot (1981). Heparin-treated peripheral blood (6 ml) was cultured in medium which contained 40 ml of RPMI 1640, 20% FCS, 100 units/ml penicillin G sodium, and 100μg/ml streptomycin, with stimulation by 1.5 ml of phytohemagglutinin solution (GIBCO) in a $CO_2$ incubator for 72 hr at 37°C. Two hundred micrograms of BrdU solution per ml of medium was added, and after 16 hr the cells were washed twice with RPMI 1640 and incubated in fresh medium containing 10μM thymidine. *In situ* hybridization was performed following the methods described by Harper and Sanders (1981), and Zabel *et al.* (1983). The chromosomes on the slides were denatured and air dried. The chromosomal DNA was hybridization with $^{3}$H-labeled probe. The slides were autoradiographed, and stained by a variation of the method of Wolff and Pelly (1974).

## RESULTS

*1) Chromosome sorting and spot blot hybridization*

Spot-blot hybridization of flow sorted chromosomes was carried out to determine the chromosomal localization of the HLUG 25 cDNA gene. GM00130B cell chromosomes stained with Hoechst 33258 were separated into 17 fractions, consisting of one to four types of chromosomes in such fractions (Fig. 1). Eighty thousand chromosomes of each type were sorted and hybridized with a $^{32}$P-labeled HLUG 25 cDNA probe. As shown in Fig. 2, only fraction Ab, containing chromosome 1, revealed a significant signal. These results indicate that the bilirubin UDPGT gene maps to chromosome 1.

*2) In situ hybridization*

Silver grains of autoradiography for a mapping were counted if the grains were on chromosome or touching a chromatid. At total of 731 grains on chromosomes of 396 cells were analyzed (Figs. 3 and 4). The chromosomal 1q21-q23 region had 38 grains (5.2% of the total number of grains, 30.6% of the grains on chromosome 1). Figure 4 shows the grain distribution over chromosome 1. There is a significant accumulation of grains in the 1q21-q23 region, especially on 1q22. This was consistent with the results of mapping by spot-blot hybridization.

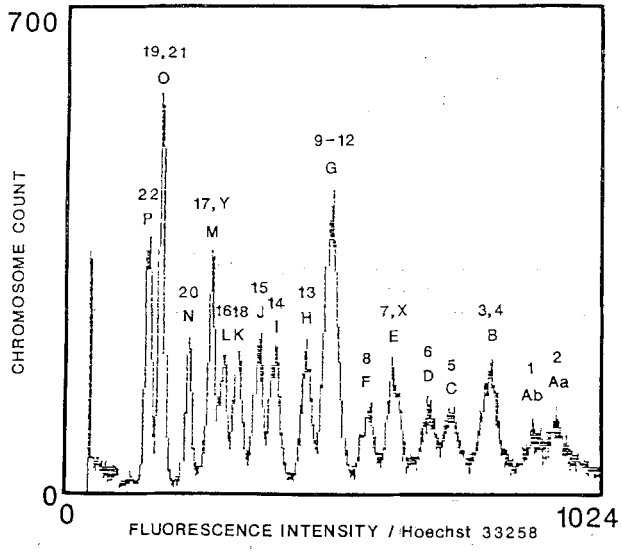


Fig. 1. A flow karyotype of human lymphoblastoid cell line GM00130B (46, XY). Chromosomes were prepared by the polyamin/digitonin method, stained with Hoechst 33258, and analyzed by a FACS440 cell sorter.

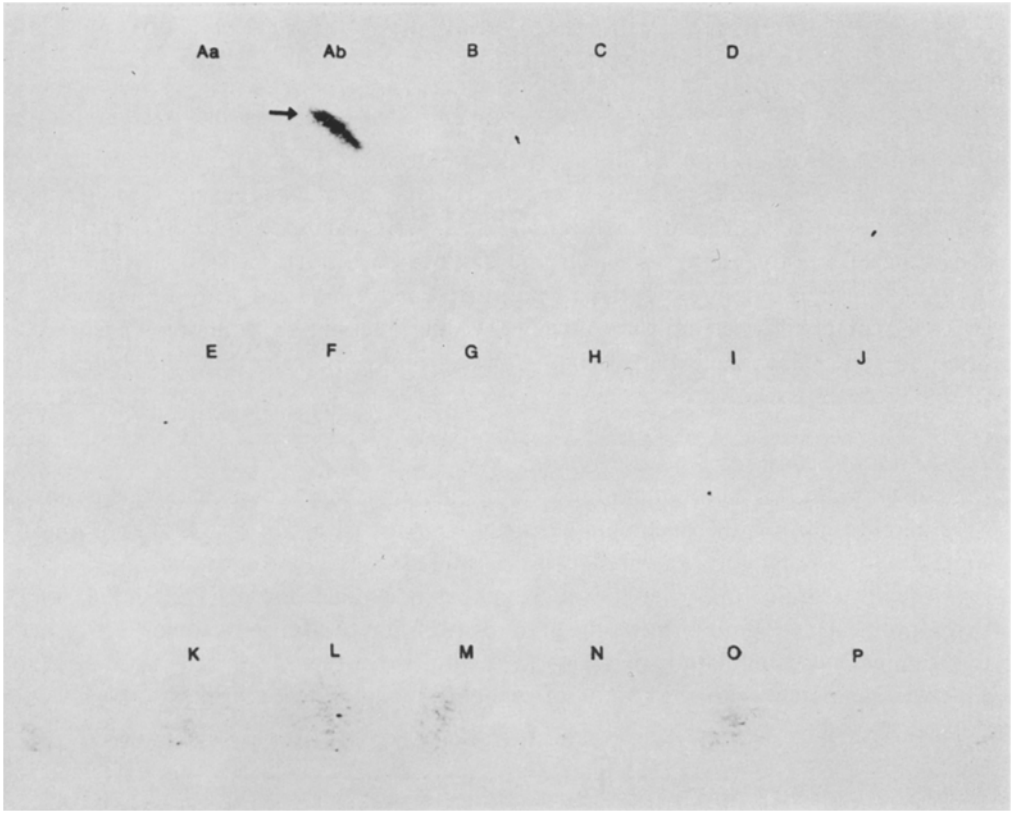


Fig. 2. Chromosomal assignment of the bilirubin UDPGT gene by spot-blot hybridization. Eighty thousand chromosomes of each type in each group shown in Fig. 1 were directly sorted onto nitrocellulose filter disks. Hybridization was carried out with the HLUG 25 cDNA probe.

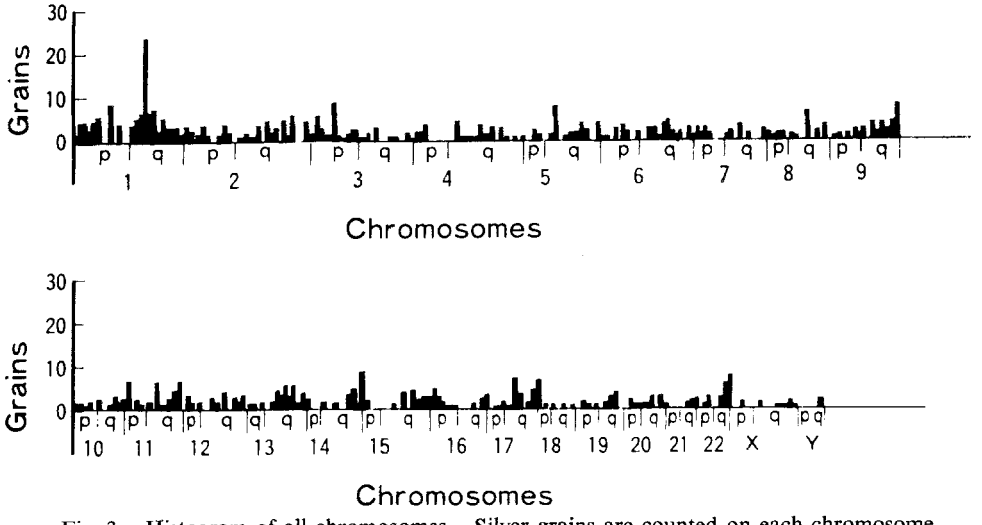


Fig. 3. Histogram of all chromosomes. Silver grains are counted on each chromosome and their bands. The long arm of chromosome 1 had the highest peak.

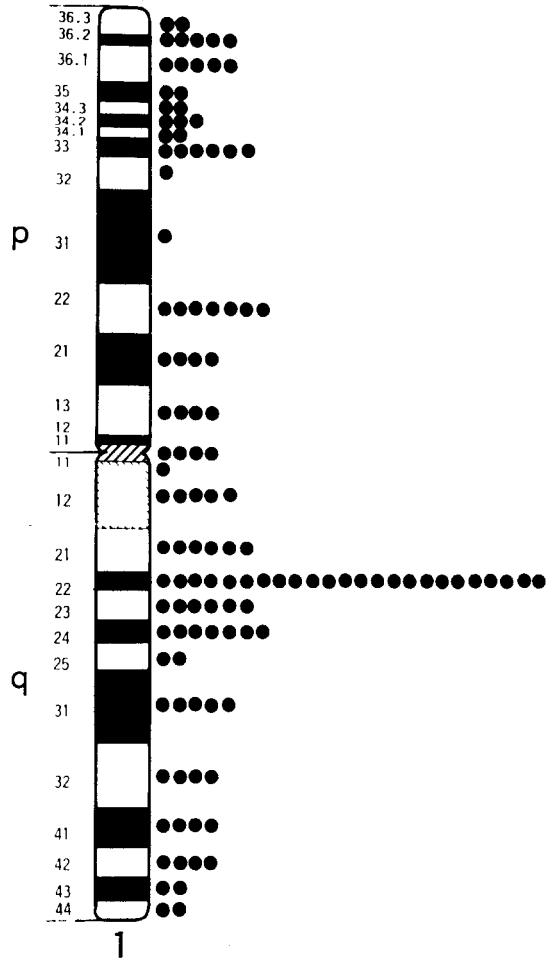


Fig. 4. Ideogram of chromosome 1. 1q21-q23, especially the 1q22 region has the greatest number of silver grains.

## DISCUSSION

Hepatic bilirubin UDPGT is responsible for the enzyme deficiency of Crigler-Najjar syndrome and/or Gilbert's syndrome. Its gene locus was assigned here in the 1q21-q23 region by spot-blot hybridization of sorted chromosomes and by *in situ* hybridization.

UDPGT has several isoenzymes paralleling differences of substrates. The hepatic phenol UDP-glucuronosyl transferase (GNT 1) gene was assigned to chromosome 2 by somatic cell hybridization (Harding *et al.*, 1990), but the specific region of GNT 1 determined. Isoenzyme gene loci are not always in the same region of chromosomes. UDP-glucose pyrophosphorylase 1 (UGP 1) and more than 27

genes have been assigned (HGM10, 1989) to the 1q21-q23 region. UGP 2 is an isoenzyme of UGP 1, and had been assigned to chromosome 2 (Shows *et al.*, 1978), as in the cases of bilirubin UDPGT and GNT 1 have in reported. These same distribution of loci for isozymes may reflect chromosomal evolution and gene duplication. As for chromosome deletion of 1q21-q23, no patient with persistent jaundice caused by a decrease in bilirubin UDPGT enzyme activity have been reported.

## REFERENCES

Black, M. and Billing, B.H. 1969. Hepatic bilirubin UDP-glucuronyl transferase activity in liver disease and Gilbert's syndrome. *New Engl. J. Med.* **280**: 1266–1271.

Dutrillaux, B. and Viegas-Pequignot, E. 1981. High resolution R- and G-banding on the same preparation. *Hum. Genet.* **57**: 93–95.

Harding, D., Jeremiach, S.J., Povey, S. and Burchell, B. 1990. Chromosomal mapping of a human phenol UDP-glucuronosyltransferase, GNT1. *Ann. Hum. Genet.* **54**: 17–21.

Harper, M.E. and Saunders, G.F. 1981. Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma* **83**: 431–439.

HGM10. 1989. Tenth International Workshop of Human Gene Mapping, New Haven Conference (1989) *Cytogenet. Cell Genet.* **51**: 1–4.

Jackson, M.R., MaCarthy, L.R., Harding, D., Wilson, S., Coughtrie, M.W.H. and Burchell, B. 1987. Cloning of a human liver microsomal UDP-glucurunosyltransferase cDNA. *Biochem. J.* **242**: 581–588.

Lebo, R.V., Tolan, D.R., Bruce, B.D., Cheung, M.-C. and Kan, Y.W. 1985. Spot-blot analysis of sorted chromosomes assigns a fructose intolerance disease locus to chromosome 9. *Cytometry* **6**: 478–483.

Minoshima, S., Kawasaki, K., Fukuyama, R., Maekawa, M., Kudou, J. and Shimizu, N. 1990. Isolation of giant fragment from flow-sorted human chromosomes. *Cytometry* **11**: 539–546.

Shows, T.B., Brown, J.A., Goggin, A.P., Haley, A.A., Byers, M.B. and Eddy, R.L. 1978. Assignment of a molecular form of UDP glucose pyrophosphorylase (UGPP-2) to chromosome 2 in man. *Cytogenet. Cell Genet.* **22**: 215–218.

Wolff, S. and Perry, P. 1974. Differential giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. *Chromosoma* **48**: 341–353.

Zabel, B.U., Naylor, S.L., Sakaguchi, A.Y., Bell, G.T. and Shows, T.B. 1983. High-resolution chromosomal localization of human gene for amylase, propiomelanocortin, somatostatin, and a DNA fragment (D3S1) by *in situ* hybridization. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 6932–6936.