

Fine Assignment of β -Hexosaminidase A α -Subunit on 15q23-q24 by High Resolution In Situ Hybridization

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160 (3), 203-211 — Tay-Sachs disease results from mutation in the gene encoding
 β -hexosaminidase A α -subunit. Although some reports have suggested the locus
on 15q, we tried to determine the finer gene locus using high resolution in situ
hybridization. cDNA probe, p β H α -5, containing the full-length sequence for the
enzyme subunit, was 3 H-labeled within $1-4 \times 10^7$ cpm/ μ g of cDNA by nick-
translation. After molecular hybridization and autoradiography, prometaphases
were G-banded by Hoechst 33258, UV-exposure and Giemsa. A total of 227 silver
grains on chromosomes within 115 prometaphase spreads were analyzed. The
region 15q23-q24 had 27 grains, corresponding to 11.9% of the total grains and to
77.1% of the grains on chromosome 15. 20.9% of prometaphases were observed
with a grain at 15q23-q24. According to several previous reports, the shortest
region of overlap (SRO) of the locus has been 15q22-q25.1. Here we have assigned
the gene locus to the narrower region 15q23-q24 by high-resolution in situ hybridi-
zation, which is one of the most powerful strategy for the completion of human
gene map. — Tay-Sachs disease; β -hexosaminidase A α -subunit; human
gene mapping; high resolution in situ hybridization

Tay-Sachs disease has been found to be a genetic cerebral degeneration
disease characterized by the progressive and excessive storage of GM₂-gangliosides
especially in the brains of affected children. Okada and O'Brien (1969) described
the absence of β -D-N-acetylhexosaminidase in several organs and body fluids of
patients with Tay-Sachs disease, and it has been confirmed that the essential cause
of Tay-Sachs disease is a deficiency of β -hexosaminidase A. β -Hexosaminidase

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is a lysosomal enzyme, a glycoprotein with a molecular weight of about 130,000. Tallman et al. (1974) described the each component A and B is composed of four subunits. Srivastava and Beutler (1973) reported that using immunological methods the subunit gives two different polypeptides, i.e., α and β , and that component A is composed of α and β . Because Tay-Sachs disease occurs due to a deficiency of β -hexosaminidase A, mutation in gene encoding the α -subunit of β -hexosaminidase results in Tay-Sachs disease.

There have been four reports suggesting that the gene locus of the alpha subunit of β -hexosaminidase A is on a long arm of human chromosome 15. All of these investigators, using human-mouse somatic cell hybrid method, assigned the gene locus by linkage analyses among some enzymes, whose gene loci had already been determined. Three of those reports described the gene locus of β -hexosaminidase A α -subunit as located on 15q22-qter, while the rest suggested it on 15q21-qter, and so the narrowest region from these results was 15q22-qter. The gene dosage effect of β -hexosaminidase A activity of patients with Tay-Sachs disease excluded the gene locus from two regions, 15pter-q14 and 15q25.1-q26.3. Therefore the shortest region of overlap (SRO) of the gene locus of β -hexosaminidase A α -subunit has finally been narrowed to 15q22-q25.1.

The method of in situ hybridization firstly described by Gall and Pardue (1969) can "virtually" give the site-specific assignment of human genes. It now allows identification of low- and single-copy genes by using nick-translation or multiprime DNA labeling and with the high molecular weight polymer dextran sulfate. Furthermore, the incorporation of high resolution banding or replication G-banding into this method has extended the power of site-specific determination of in situ hybridization.

Using high resolution in situ hybridization, we tried to determine the gene locus of β -hexosaminidase A α -subunit to a greater degree of precision than that of the SRO from the previous reports, i.e., 15q22-q25.1.

MATERIALS AND METHODS

Chromosome preparation. The replication G-banding technique described by Dutrillaux and Viegas-Pequignot (1981) was used except for minor modifications for the blood culture (Nakai et al. 1986). The heparin-treated peripheral blood (6 ml) of a normal human male was cultured in the complete medium which contained 40 ml of RPMI-1640, 10 ml of 20% fetal bovine serum, 100 units/ml penicillin and 100 mg/ml streptomycin, with the stimulation of 1.5 ml of phytohemagglutinin (PHA; Gibco, Gaithersburg, MD, USA) in a CO₂ incubator at 37°C for 72 hr. 200 μ g of Millipore filtered BrdU solution (Sigma, St. Louis, MO, USA) per ml of the complete medium was added and incubated in the same way, and after 16 hr the cells were washed twice with RPMI-1640 and incubated in fresh complete medium containing 10 μ M of Millipore filtered solution of thymidine (Sigma). After approximately 6.0 hr, the cells were centrifuged and the pellets were resuspended in 6.0 ml of 0.075 M KCl at 37°C for 20 min of hypotonic treatment. The cells were fixed with Carnoy fixative, washed three to four times with the fresh fixative, and dropped on ice-cold slides.

The slides (2 days old) were treated with preboiled RNase A (final concentration: 100

$\mu\text{g/ml}$; Sigma) in $2\times\text{SSC}$ at 37°C for 60 min, washed twice in $2\times\text{SSC}$, dehydrated in 70% and 95% ethanol, and air dried.

cDNA probe. The cDNA clone, p $\beta\text{H } \alpha$ -5, which is the entire coding sequence of the α -chain of β -hexosaminidase (Myerowitz et al. 1985), was isolated from an adult human liver library. The cDNA insert of p $\beta\text{H } \alpha$ -5 is 1,944 base pairs long and contains a 168-base-pair 5' untranslated region, a 186-base-pair 3' untranslated region, and an open reading frame for 1,587 base pairs corresponding to 529 amino acids.

Radiolabeling of cDNA probe. Nick translation was essentially as described in Rigby et al. (1977). The 100 μl reaction mixture contained 1 μg of cDNA, three labeled nucleotides (^3H -dATP at 54.9 Ci/mmol, ^3H -dCTP at 51.0 Ci/mmol, and ^3H -TTP at 117 Ci/mmol, New England Nuclear, Boston, MA, USA; 1 Ci = $37\times\text{GBq}$), 10 μl of unlabeled dGTP, 2 μl of enzyme mixture, i.e., DNaseI and PolymeraseI (Nick Translation Kit; Takara, Kyoto), and 1 μl of bovine serum albumin (1 mg/ml) in $10\times$ Nick Translation Buffer. The reaction mixture was incubated in a water bath at 14°C , and the cDNA probe was labeled within a range of $1\text{--}4\times 10^7$ cpm/ μg of cDNA. The ^3H -labeled cDNA probe was separated from unincorporated nucleotides, and precipitated by the sodium acetate-ethanol method. Approximately 80% of the total label incorporated into the cDNA probe was recovered. The 1 ml of hybridization mixture contained 90 μl of the ^3H -labeled cDNA probe, 0.5 ml of 50% deionized formamide, 10 μl of sonicated salmon sperm DNA (10 mg/ml), 0.25 ml of 40% dextran sulphate (Wahl et al. 1979), 10 μl of 10% SDS, 40 μl of 1 M phosphate buffer (pH 6.5), 50 μl of $20\times\text{SSC}$, and 50 μl of $20\times\text{SSC}$ - $20\times$ Denhardt's.

In situ hybridization. In situ hybridization was performed following the methods described by Harper and Saunders (1981), and Zabel et al. (1983). The chromosomes on the slides were denatured in 70% formamide/ $2\times\text{SSC}$ (pH 7.0) at 70°C for 2 min, followed by quick dehydration sequentially in 70% and 95% ethanol at -20°C and air dried. The hybridization mixture was heated at 70°C for 5 min, frozen in a dry ice bath, and after thawing placed on slides under a siliconized coverslip (100 μl with 20 ng of denatured cDNA probe per slide). Incubation was carried out in a $2\times\text{SSC}$ saturated environment in FALCON Petri dishes sealed with parafilm at 37°C for 24 hr. And then, the slides were washed by the method of Zabel et al.

Autoradiography. The slides were dipped for approximately 1 sec in NTB 2 emulsion (Kodak)/ H_2O 1:1 (vol/vol) at 42°C . After being air dried in the dark for 1 hr, the slides were placed in a light-tight box with Drierite for 5–14 days at 4°C . After 5–14 days, the slides were developed in Kodak D-19, diluted 1:1 with water, for 4 min at 15°C , fixed in Kodak fixer for 5 min at 15°C (still in the dark), and rinsed in water for 15 min (in light) followed by air drying for 24 hr.

Staining (G-banding). A variation of the method of Wolff and Perry (1974) was used. The slides were first stained in Hoechst 33258 (1 $\mu\text{g/ml}$ of $2\times\text{SSC}$) for 15 min, rinsed with water, air dried, and then exposed to long-wave ultraviolet (360 nm) for 1 hr (the $2\times\text{SSC}$ -covered slides were kept 20 cm from the UV source). After being rinsed and air dried, the slides were stained for 12 min with a 5% Giemsa solution in Sorensen's buffer, pH 6.8, rinsed with water and air dried.

RESULTS

Cell synchronization with BrdU consistently yielded a high percentage of prophase and prometaphase mitoses. About 60–70% of all mitoses were one of these stages, and, after banding, about 550 bands per haploid set of chromosomes were clearly and almost consistently visible. Almost all of mitoses were G-banded; the rest gave an R-banding pattern. The procedure used gave a direct visualization of silver grains from the labeled probe together with the distinct



Fig. 1. Prometaphase spread showing two grains on band q23 of chromosome 15.
 (a) shows G-banded pattern using a green interference filter.
 (b) shows grains more clearly without a filter.

G-banding chromosome pattern, using an ordinary light microscope. The banding pattern of the prometaphase spreads was best observed when a green interference filter was used, but, the silver grains were best visualized without this filter (Fig. 1).

One hundred fifteen prometaphases were analyzed, and 227 silver grains were scored with an average number of 2.0 grains/prometaphase spread (Table 1). Of these grains, 35 were on chromosome 15. The chromosomal region 15q23-q24 had 27 grains, which corresponded to 11.9% of the total grains and to 77.1% of those

TABLE 1. *Chromosomal localization of β -hexosaminidase A α -subunit by high resolution in situ hybridization*

Total number of prometaphase analyzed	115
Total number of grains counted	227
Average number of grain/prometaphase (227/115)	2.0
Number of grains on chromosome 15	35
Number of grains at 15q23→q24	27
% of total grains at 15q23→q24 (27/227)	11.9
% of prometaphases with a grain at 15q23→q24 (24/115)	20.9
% of grains on chromosome 15 at 15q23→q24 (27/35)	77.1

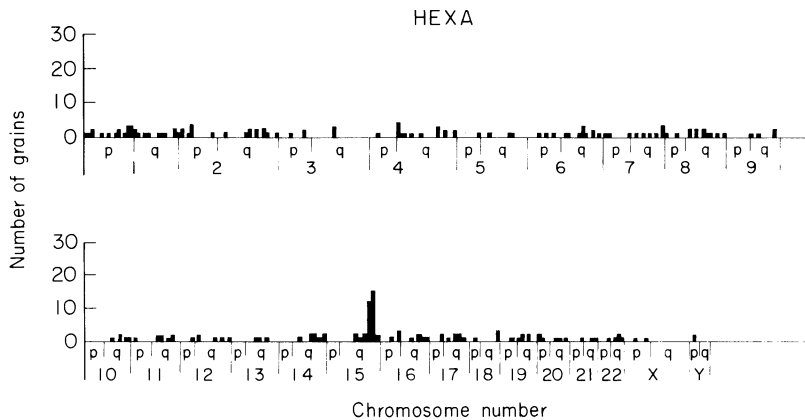


Fig. 2. Grain distribution on all chromosomes.

on chromosome 15. 20.9% of prometaphases were observed with a grain at 15q23-q24. No other sites were labeled above background. Fig. 2 shows the distribution of grains on all chromosomes (the x axis represents the chromosomes in their relative size proportion and the y axis gives the number of silver grains).

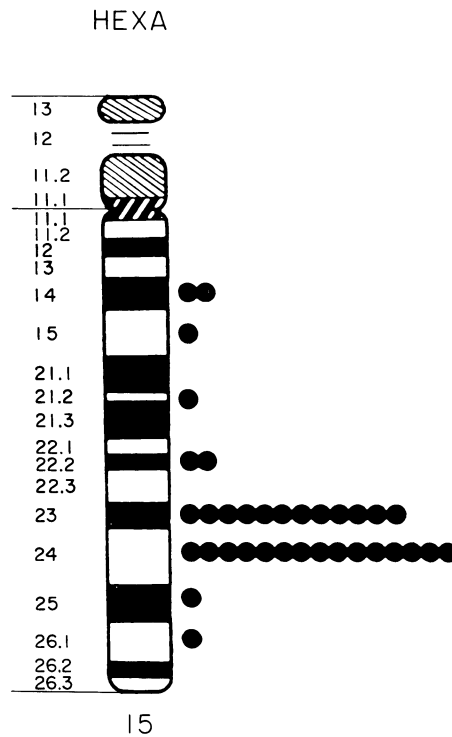


Fig. 3. Ideogram of chromosome 15 and silver grain distribution on it. The region 15q23-q24 had the highest peak, suggesting the gene locus of β -hexosaminidase A α -subunit.

A distal region of the long arm of chromosome 15 has a significant amount of labeling in comparison to the unspecific background grains found on all chromosomes.

The ideogram of chromosome 15 shows a very significant accumulation of silver grains on the region 15q23-q24, suggesting the gene locus of β -hexosaminidase A α -subunit (Fig. 3).

DISCUSSION

To assign human genes to chromosomes, regions of chromosomes and (sub) bands of chromosomes, there are four major types of strategies, except for a few biochemical strategies representing other specialized procedures, each with specific methodologies: (1) family studies; (2) somatic cell hybridization; (3) Southern hybridization combined with somatic cell hybrids; (4) in situ hybridization.

Gene mapping using family study data requires the segregation of traits with an identifiable chromosome, and allelic variation of both the chromosome variation and the trait are necessary. This strategy does also have such other drawbacks as the impossibility of controlled mating and the very long generation time of humans. The developments of the somatic cell hybridization and Southern hybridization combined with somatic cell hybrids for mapping human genes have resulted in an explosive increase in the number of human genes assigned to specific chromosomes or regions of chromosomes, but they have had three problems: (1) instability of the numbers of retained human chromosomes in hybrid cells and an associated low detection sensitivity; (2) unreliability of human chromosomes present in hybrid cells because of frequent human chromosome rearrangement during the cell culture; (3) determination limited to chromosomes only or at best to chromosomal regions.

The application of recent high resolution banding or replication G-banding techniques has extended the power of in situ hybridization. BrdU added to a cell culture blocks synthesis in the middle of S-phase in the cell cycle and the cells become synchronized at this point. By release of the block and continued culture in a thymidine-enriched medium, the synchronized population of cells can be harvested without the use of colcemid. Subsequent Hoechst 33258-UV exposure-Giemsa staining results in replication-banded chromosomes where BrdU-incorporated regions (early replicating) appear as pale bands and thymidine-incorporated regions (late replicating) stain darkly.

This high resolution in situ hybridization has several merits in human gene mapping. First, the replication G-banding corresponds very closely to trypsin G-banding except for the late replicating x chromosome. Second, the obtained chromosomes are almost consistently prometaphases with high resolution banding. Thirdly, because of the hybridization between cDNA probe and normal human chromosomal DNA in this high resolution in situ hybridization, misinterpretation derived from chromosomal rearrangement can be absolutely avoided. Fourthly,

this advanced technique allows a finer identification of bands of chromosomes and occasionally even of subbands of chromosomes. There is, however, one principal problem with this method: for the identification of chromosomes on which silver grains locate, both high quality chromosome banding and skillful identification are necessary.

Using human-mouse somatic cell hybridization several investigators have assigned the gene locus of β -hexosaminidase A α -subunit to the long arm of human chromosome 15. Lalley et al. (1974) have found that a gene involved in hexosaminidase A expression was linked to the genes coding for mannosephosphate isomerase and pyruvate kinase-3. Gilbert et al. (1975) have also been able to establish a syntenic relationship between the gene locus responsible for the expression of hexosaminidase A and those responsible for mannosephosphate isomerase and pyruvate kinase-3. Chern et al. (1977) reported that concordant segregation of the expression of human hexosaminidase A α -subunit, mannosephosphate isomerase, and pyruvate kinase-3 had been observed in somatic cell hybrids between either thymidine kinase-deficient mouse cells or thymidine kinase-deficient Chinese hamster cells and human leukocytes carrying a translocation of q22-qter of chromosome 15 to chromosome 17. All of these reports suggested that the gene locus of β -hexosaminidase A α -subunit is located on the region q22-qter of human chromosome 15. On the other hand, Hellkuhl et al. (1978) suggested that the genes for β -hexosaminidase A α -subunit, mannosephosphate isomerase, pyruvate kinase-3, and mitochondrial isocitrate dehydrogenase are localized to the region 15q21-qter. Furthermore, by assays of β -hexosaminidase A and B activity on serum from three patients with different rearrangements of chromosome 15, Magenis et al. (1979) excluded the gene locus from the regions 15pter-q14 and 15q25.1-q26.3. Therefore, the SRO of the gene locus of β -hexosaminidase A α -subunit derived from all of these reports has been the region q22-q25.1 of human chromosome 15.

By high resolution in situ hybridization, silver grains have accumulated very significantly and remarkably on the region q23-q24 of chromosome 15. Therefore, we have assigned the gene locus of β -hexosaminidase A α -subunit to the region q23-q24 of human chromosome 15. This result is, to the best of our knowledge, the narrowest region yet identified in gene mapping of human β -hexosaminidase A α -subunit, and gives the more detailed localization than does the previous SRO, i.e., the region 15q22-q25.1.

A precise and detailed knowledge of the specific localization of individual human genes on chromosomes is basic for understanding the organization of the human genome, the function of individual genes and of the total genome, and the regulation of gene expression. Such information on organization, function, and expression of the human genome is essential for understanding all stages of normal and abnormal development and physiology. In addition, an understanding of genetic diseases is gained by knowing where the affected genes are located and

their relationships to other and/or adjacent genes, which can be provided only by a fine human gene map. This gene map will yield information beneficial to the isolation and purification of affected genes in diseases without established causes, and so contribute not a little toward the discovery of the causes of these diseases. Furthermore, gene walking on the human gene map will gradually determine the gene fragments of specific chromosomes and will allow us to determine the full-length nucleotide sequence of each chromosome. A knowledge of the gene map of each human chromosome will also be useful in prenatal diagnosis and in understanding the evolution of humankind through comparative gene mapping.

High resolution in situ hybridization is the most powerful strategy for the completion of the human gene map; it has great usefulness for both basic and clinical medicine, and presents much information about embryology, differentiation, production, malignancies, genetic diseases and so on, as described above.

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