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Huntington Disease-Linked Restriction Fragment Length Polymorphism Localized within Band p16.1 of Chromosome 4 by In Situ Hybridization

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SUMMARY

A 5.5-kilobase (kb) single sequence DNA fragment (G8) reveals the DNA polymorphic locus *D4S10* on Southern blot analysis. This locus is closely linked to Huntington disease and has been mapped to chromosome 4 short arm using human-mouse somatic cell hybrids, and specifically to chromosome 4 band p16 using DNA from individuals with deletions of chromosome 4 short arm who exhibit Wolf-Hirschhorn syndrome. With in situ hybridization techniques, we have confirmed the location of *D4S10* on chromosome 4 and further localized it within band p16 utilizing five patients, four with overlapping chromosome 4 short-arm aberrations.

The DNA segment G8 was hybridized to the mataphase chromosomes of the five patients. Two of them have different interstitial deletions of one of the chromosome 4 short arms (TA and BA), two have different chromosome 4 short-arm terminal deletions (RG and DQ), and one has a normal male karyotype. By noting the presence or absence of hybridization to the partially deleted chromosomes with known precise breakpoints, we were able to more accurately localize probe G8 to the distal half of band p16.1 of chromosome 4.

INTRODUCTION

Huntington disease (HD) is a late-onset autosomal dominant disorder characterized by degeneration of the nervous system [1–3]. Central nervous system

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development is normal but premature neuronal cell death occurs. The disease is characterized by choreiform movements, personality changes, loss of memory, and mood shifts. The impact of the disease on families is devastating. A person with the HD gene will usually appear normal during the early years. Manifestation of symptoms of the disease may not occur until the third, fourth, or even fifth decade of life; by this time the defective gene may have been passed on to offspring. The disease progresses for 10–20 years until the affected individual is completely disabled.

The primary biochemical defect and specific gene involved in HD are unknown. Recently, however, recombinant DNA methods have been employed in an attempt to localize the HD gene.

Gusella et al. in 1983 [4] reported isolation of a human restriction fragment (G8), which revealed a DNA polymorphic locus, *D4S10,* closely linked to the HD gene. This locus was mapped to chromosome 4 short arm by use of human-mouse somatic cell hybrids [4] and to the terminal band of chromosome 4 short arm by heterozygosity and dosage analysis of patients with Wolf-Hirschhorn syndrome [5]. It is anticipated that isolation of the disease gene will be possible when specific localization of the gene to chromosome 4 is accomplished.

To further map the *D4S10* polymorphic locus to the terminal short arm of chromosome 4, we chose five subjects for in situ hybridization studies using probe G8. These subjects include a normal male, two with chromosome 4p terminal deletions (at bands 4pl6.1 [DQ] and 4p15.3 [RG]), and two with interstitial deletions (from p16.1→p14 [TA] and p16.1→p15.1 [BA]). Although three of the subjects have breaks in p16.1, their breakpoints are not identical. The patient, RG, with the large terminal deletion, 4p15.3→4pter, has typical Wolf-Hirschhorn syndrome with profound mental and growth retardation as well as facial features of hypertelorism, elfin ears, and "Greek-warrior helmet" configuration of the nose [6–8].

The patient with the smaller terminal deletion (DQ) has less severe mental and growth retardation but has similar facies. The interpretation of breakpoints in the chromosome 4 of this patient was difficult, and an alternate interpretation of a small interstitial deletion is possible. The phenotype as well as chromosome appearance, however, are most compatible with the interpretation given. The patient with the small interstitial deletion, p16.1→p15 (BA), has clinical features similar to the patient described by Francke et al. [9] including normal stature, moderate mental retardation, and long facies, while the subject (TA) with the large interstitial deletion has features that appear to be a combination of both those of interstitial deletion and terminal deletion. He has no hypertelorism, has normal ears, and manifests growth retardation and severe mental retardation. His deletion includes more of band p16.1 than that of BA. Chromosomes 4 of these subjects at the 750–850 band stage are shown in figure 1.

We hypothesized that presence or absence of hybridization to the abnormal chromosome 4 utilizing these patients with overlapping deletions would allow high resolution mapping of the *D4S10* polymorphic locus within a single chromosome band.

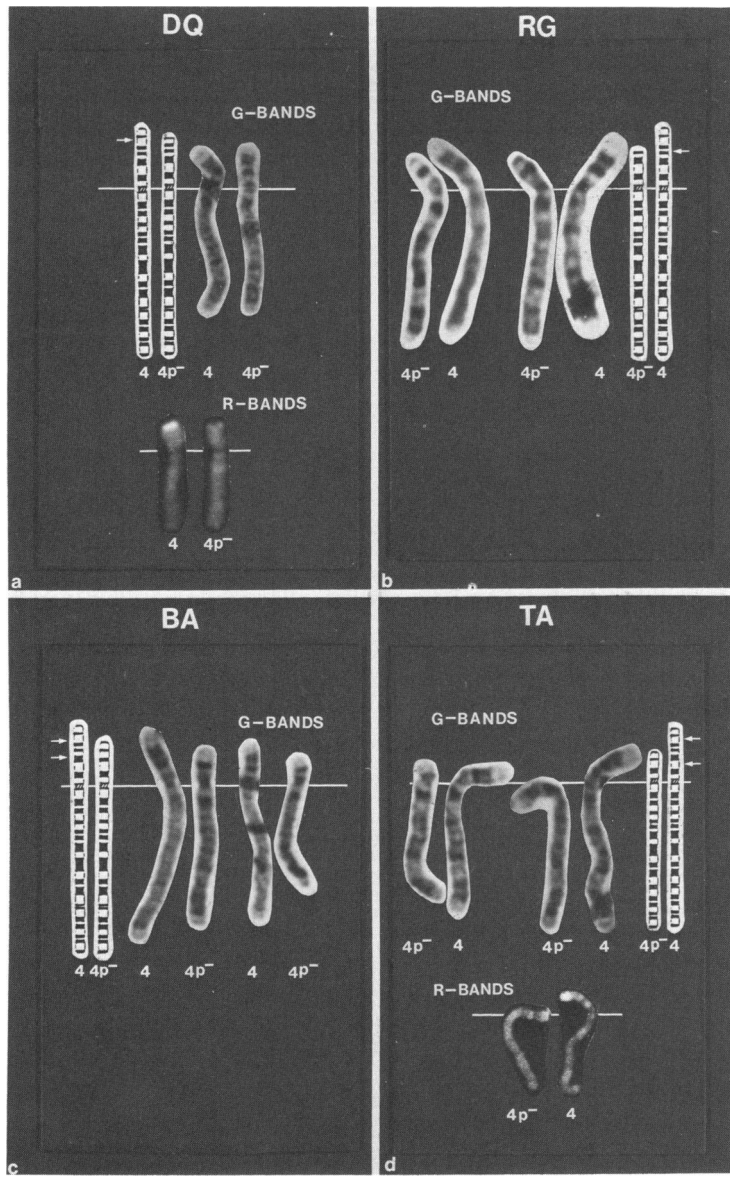

FIG. 1.—Composite of high-resolution chromosomes 4 from the patients with deletions of 4p. Ideograms modified from ISCN, 1985 [10]. *a*, G-banded (*top*) and R-banded (*bottom*) chromosomes 4 from DQ. The G-banded chromosomes are at approximately the 700-band stage; there is a deletion of the right hand 4 at p16.1. *Arrow* on ideogram points to breakpoint. *b*, G-banded chromosomes 4 at 700–800-band stage from RG. Left-hand chromosomes 4 have deletion at 4p15.3. *Arrow* on ideogram points to breakpoint. *c*, G-banded chromosomes 4, at 850-band level, from patient BA; there is an interstitial deletion of bands 4p15.1→16.1, indicated by *arrows*. *d*, G- and R-banded chromosomes 4 from TA. There is a large interstitial deletion from 4pl4→p16.1 (*arrows* on ideogram point to breakpoints). R-banded chromosomes (*below*) show presence of bright distal euchromatin in both homologs, but less of this material (p16) is present in the deleted chromosomes. This indicates that the proximal portion of p16 is missing.

MATERIALS AND METHODS

The DNA subclone pK082 of the G8 probe was radioactively labeled by nick-translation using [^{3}H]TTP and [^{3}H]CTP to a specific activity of 2×10^7 dpm/μg. In situ hybridization to metaphase chromosomes on microscope slides was carried out according to Harper and Saunders [11]. Each slide was incubated at 37°C for 12 hrs with 100 μl of probe solution containing 0.2 μg/ml labeled probe DNA. Slides were coated with Kodak NTB 2 liquid emulsion (diluted 1:1) and placed in the dark for 6–10 days at 4°C. Hybridized slides were R-banded using chromomycin A_3/distamycin A, and the locations of exposed silver grains on the chromosomes of 200 cells were recorded for each subject. A combination of fluorescent and transmitted illumination was used to identify the chromosomes and detect the grains. Background values of silver grains average 17 grains/1,000× field, but only an average of two grains/cell were found on chromosomes. Slides were then destained and restained with Wright stain so that the location of silver grains could be photographed.

RESULTS

In 44 of 200 cells examined from the normal male subject, probe hybridization was evident on the distal end of chromosome 4 short arm. In figure 2, left, chromosomes 4, at the 850-band stage, from this subject are shown; the banding pattern of these chromosomes is normal. The distribution of grains from 200 cells is illustrated by dots on the accompanying ideogram (right) in figure 2. There is a clear excess of grains located on the distal chromosome 4 short arm, an indication that D4S10 maps to that region.

In 24 of 200 cells scored from patient DQ, grains were present on the distal portion of chromosome 4 short-arm band p16. These grains were only on the normal chromosome 4, with the exception of one cell, in which a grain was located on the portion of p16 still present in the deleted 4. Approximately half the number of cells had silver grains on chromosome 4 as compared to the cells from the normal individual. In figure 3, a representative metaphase spread from DQ is pictured. A grain is present on the normal chromosome 4 short arm.

No significant hybridization was observed to the short arm of the other abnormal chromosomes 4 that were missing the distal portion of band p16.1 (RG and TA); however, hybridization to the normal chromosomes was observed. Thirty-eight of 240 cells from patient BA exhibited hybridization to the terminal short arm of chromosome 4; half the cells had grains on the normal 4 and half on the deleted 4, indicating the *D4S10* site was still present in the abnormal 4. The abnormal 4 in this case contained the distal half but not the proximal half of 4p16.1. The distribution of grains on chromosomes 4 from the four patients with deletions is shown in figure 4. Grain counts from all subjects are summarized in table 1.

The breakpoints and the overlapping segments of the abnormal chromosomes 4 are illustrated in figure 5. In particular, the deleted segments from TA and DQ, both of which must contain D4S10, are differentially shaded so that the overlap region is clear. The combined data suggests that the *D4S10* site must be located in the overlap region, within the distal half of band p16.1.

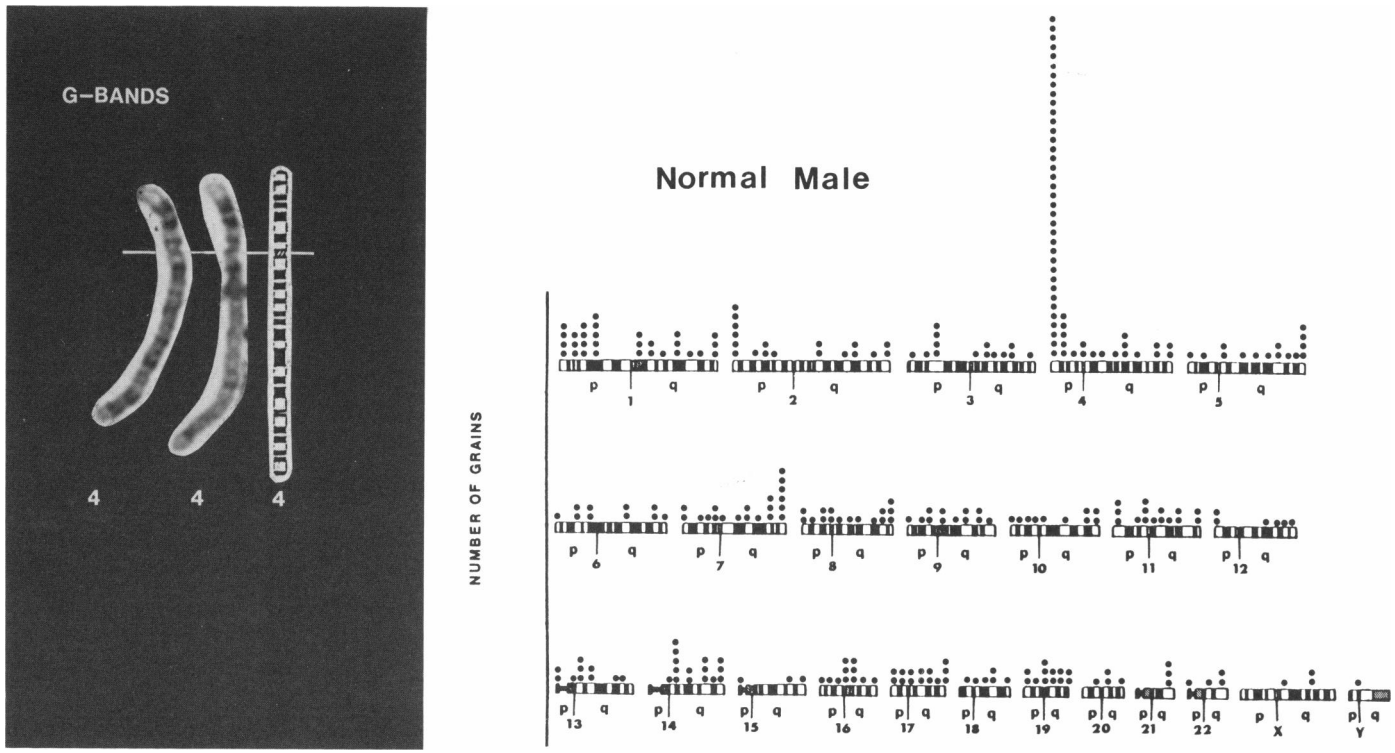

FIG. 2.—*Left:* Composite high-resolution G-banded chromosomes 4 from a normal male. Chromosomes appear normal. *Right:* Grain distribution diagram illustrates the concentration of grains on distal 4p found in 200 cells. Chromosomes are placed horizontally to facilitate placement of dots. *Each dot* represents a grain found at that particular location on a chromosome.

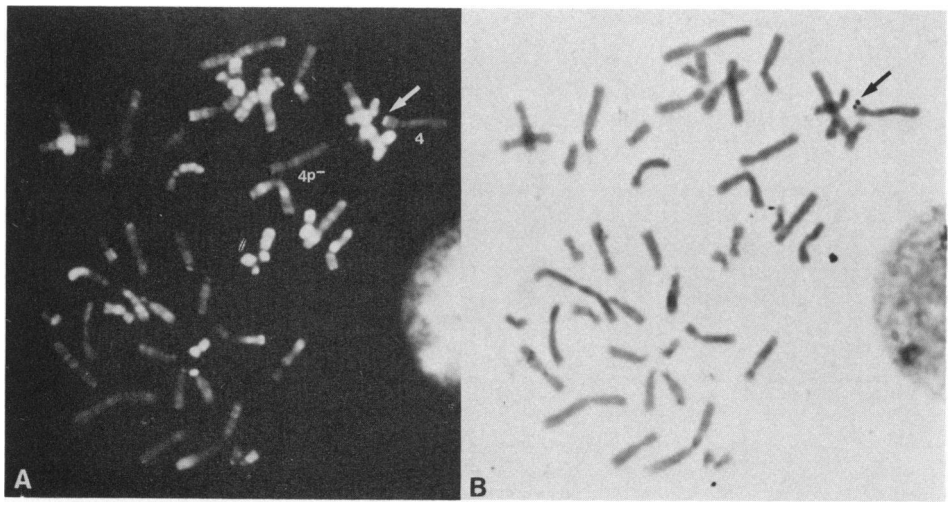

FIG. 3.—A representative metaphase from patient DQ, which has been hybridized with G8, (*A*) R-banded, and, then, (*B*) restained with Wright stain. *Arrows* indicate silver grains over the distal short arm of the normal chromosome 4 (*B*) and the corresponding location on the R-banded photo (*A*).

DISCUSSION

At least 16 different methods have been successfully used to map genes onto chromosomes [12]. The various methods have provided complementary as well as confirmatory information. The "smallest region of overlap" between results of various methods of mapping a gene often has permitted precise localization. In situ hybridization of unique sequence DNA maps the sequence to a chromosomal region, but grain scatter generally prevents intraband mapping. The use of selected multiple overlapping deletions or rearrangements of the involved chromosomal region allows very precise localization. A limiting factor in the precision of localization is breakpoint interpretation of the abnormal chromosomes. Multiple staining techniques in addition to prometaphase stage chromosomes may be necessary.

TABLE 1

RESULTS OF G8 HYBRIDIZATION TO 4p15.3→4pter

PATIENT	No. CELLS SCORED	PERCENTAGE OF CELLS WITH HYBRIDIZATION		
		Normal 4	Deleted 4	Total
Normal male	200	22	...	22
RG	200	12	< 1	12
DQ	200	12	< 1	12
TA	200	11	0	11
BA	240	8	8	16

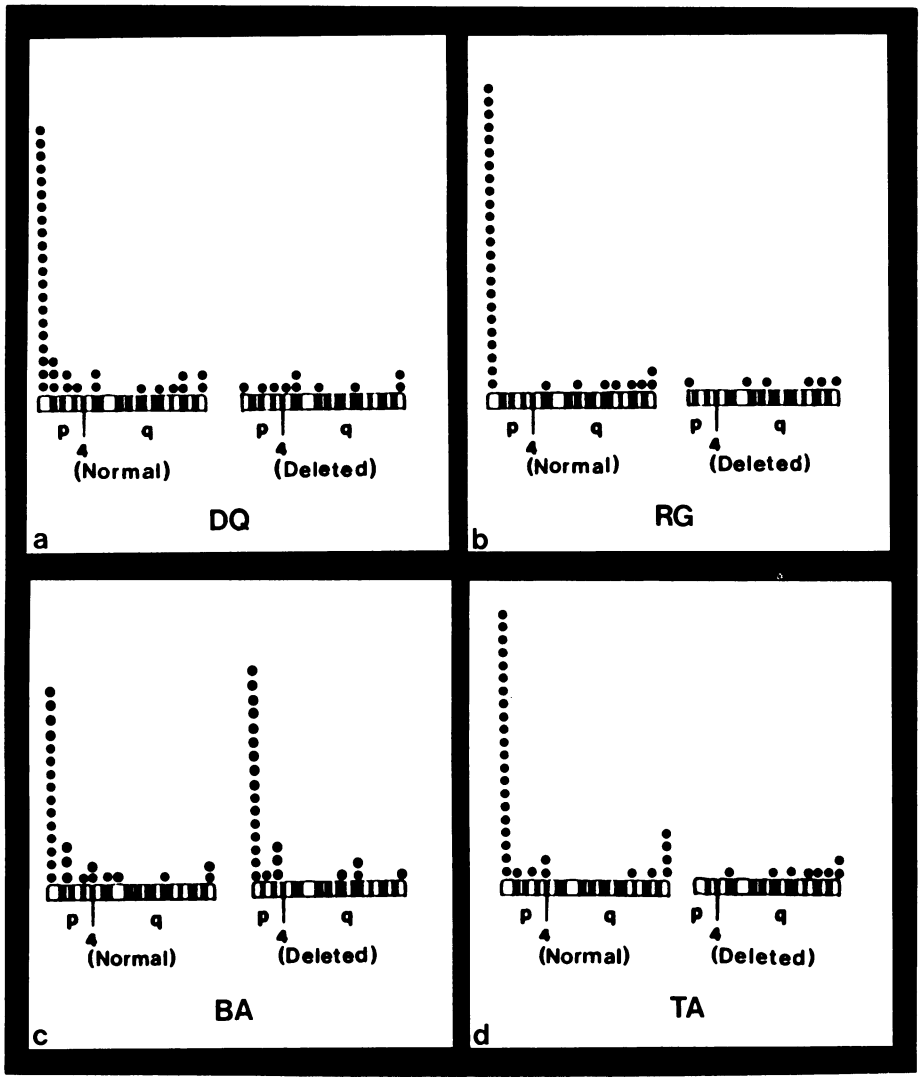

FIG. 4.—Grain distribution diagrams show the concentration of grains on distal 4p of the four patients studied with 4p deletions. *Each dot* represents one grain found at that particular location on a chromosome. The deleted chromosomes 4 (*a*, *b*, and *d*) did not hybridize with G8 with the exception of BA (*c*); the abnormal 4 in this case still contained most of band p16.1.

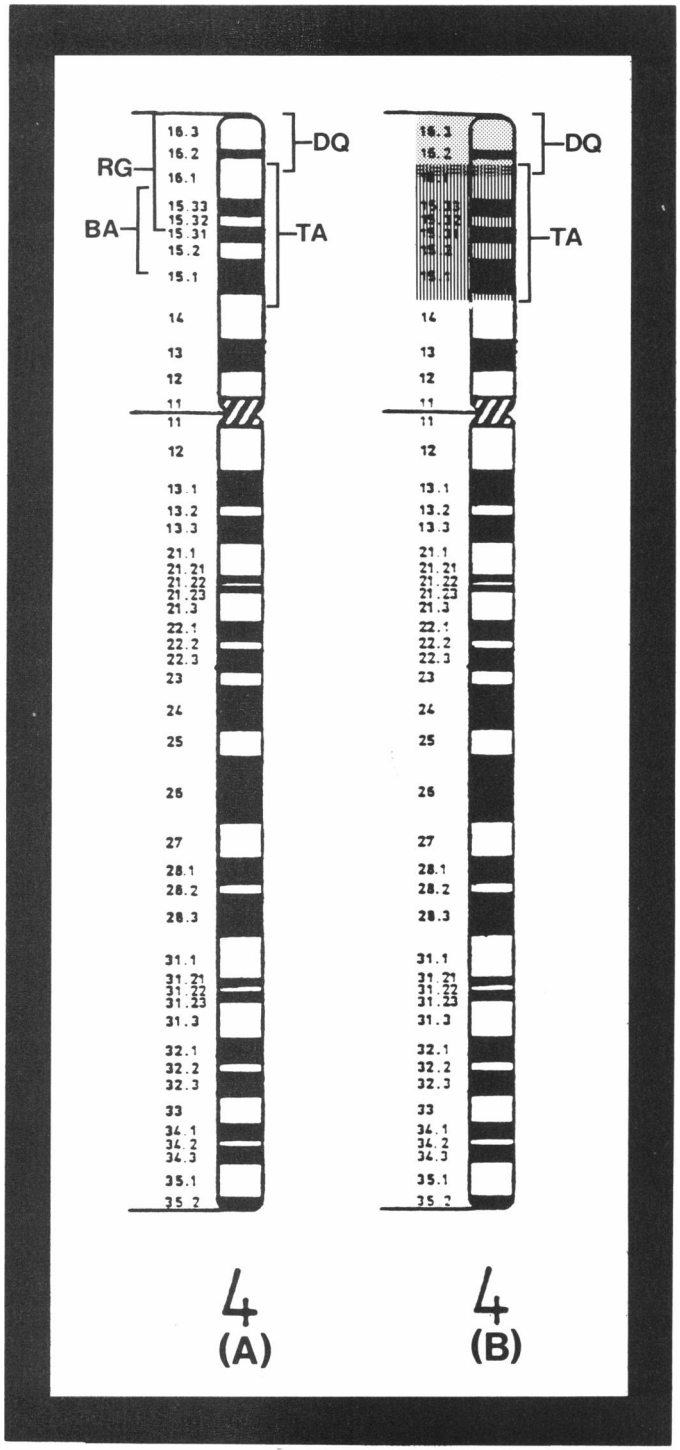

FIG. 5.—*A*, The deletions found in each of the four abnormal patients are indicated by *brackets* on the ideogram of chromosome 4 (ISCN 1985) [10]. *B*, The differential stippling emphasizes the deletions found in DQ and TA with the *cross-hatched region* of overlap illustrating the region of probe localization.

The results of this study, using multiple overlapping deletions of the short arm of chromosome 4, place the G8 locus (*D4S10*) in a narrow intraband region, the distal half of chromosome 4 high resolution band 4p16.1. The tightly linked Huntington disease locus is then also mapped either in or close to this segment.

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