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A Deletion Map of the WAGR Region on Chromosome 11

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Summary

The WAGR (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation) region has been assigned to chromosome 11p13 on the basis of overlapping constitutional deletions found in affected individuals. We have utilized 31 DNA probes which map to the WAGR deletion region, together with six reference loci and 13 WAGR-related deletions, to subdivide this area into 16 intervals. Specific intervals have been correlated with phenotypic features, leading to the identification of individual subregions for the aniridia and Wilms tumor loci. Delineation, by specific probes, of multiple intervals above and below the critical region and of five intervals within the overlap area provides a framework map for molecular characterization of WAGR gene loci and of deletion boundary regions.

Introduction

The WAGR (i.e., Wilms tumor, aniridia, genitourinary anomalies, and mental retardation) syndrome is associated with constitutional heterozygous deletions of the short arm of chromosome 11, with a common region of overlap at band p13 (Francke et al. 1979; Riccardi et al. 1980; Shannon et al. 1982). Chromosomal deletions including 11p13 have also been found in tumor tissue of individuals with Wilms tumors and no additional features of the WAGR syndrome (Kaneko et al. 1981; Douglass et al. 1985). Furthermore, in two families with familial aniridia, this condition was associated with different chromosomal translocations, both involving 11p13 (Simola et al. 1983; Moore et al. 1986). This region is therefore assumed to contain a Wilms tumor gene, as well as additional genes associated with the developmental malformations of the syndrome.

The first marker mapped to the WAGR deletion region was the catalase gene (Junien et al. 1980), and reduced levels of erythrocyte catalase activity have been observed in a number of individuals with these deletions (Ferrell and Riccardi 1981; Junien et al. 1983). By use of molecularly cloned probes, the critical region could be narrowed down to an unmapped area between the catalase and the follicle-stimulating hormone beta-subunit (FSHB) genes, estimated at several million base pairs in size (Glaser et al. 1986; Porteous et al. 1987). We have used a bank of 31 probes and six reference loci to divide the WAGR region into 16 intervals, each defined by one or more deletion boundaries. The aniridia subregion of the WAGR complex locus has been identified, and the region proximal to this locus, which contains the Wilms tumor gene, has been defined by a number of conserved-sequence DNA probes.

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Material and Methods

Cell Lines, DNAs, and Probes

Lymphoblastoid cell lines GM5518, GM6803, GM7736, and GM8785 and fibroblast cell lines GM3118, GM3808, and GM5296 were obtained from the Institute for Medical Research, Camden, NJ. Somatic cell hybrid lines for chromosomal mapping of DNA probes to chromosome 11 have been described elsewhere (Bruns et al. 1979). The deletions in the N.W., M.J., and DAR hybrid cell lines (Bruns et al. 1984; Turleau et al. 1984) are indicated in table 1. The nondeleted chromosome

Table 1

Cell Lines for the Deletion Analysis of WAGR Probes

Cell Line	Sex	Karyotypic Abnormality	Phenotype[a]
Lymphoblastoid cells:[b]			
GM 3118	F	del 11p13-p11	WA[c]
GM 3808	M	del 11p14-p11.2	WAGR
GM 5296	F	inv ins (10;11)(11pter > 11p13:: 10q21 > 10q24::11p13 > 11qter)	Coloboma right iris, microcephaly, multiple congenital malformations, neonatal death
GM 5518	F	del 11p14-p12[d]	WAR
GM 6803	M	del 11p13-p11.2	AGR
GM 7736	M	del 11p14.1-p13	AGR
GM 8785	F	del 11p14.2-p13	A, developmental delay
D.G. 85	M	t(11;22)(p13;q12.2)	A
Hybrid cells:			
N.W.	M	del prox11p14-dist11p12	WAGR
M.J.	F	del prox11p14-p13	WAR
DAR	M	del 11p13	WG
Leukocytes:			
BC	M	del 11p14-p13	AGR
C.L.	F	del 11p13	WA

[a] W,A,G and R = Wilms tumor, aniridia, genitourinary anomalies, and mental retardation, respectively.
[b] Information for GM cell lines was taken from the NIGMS catalog, 1986 ed.
[c] No information on mental status was available on this individual.
[d] Complex rearrangement of 9 and 11, resulting in a net deletion of 11p14-p12 (de Martinville and Francke 1983).

11 homologues were not present in these three hybrids. Cell line D.G.85 had been established from patient IV-4 of Moore et al. (1986). After informed consent was obtained, peripheral blood leukocytes for DNA isolation were obtained from C.L. (Russell and Weisskopf 1986) and from BC (table 1).

The catalase cDNA clone C2–2 containing a 2.0-kb insert has been described elsewhere (Bruns et al. 1984). Probe pS8–1 (HVBS1; Rogler et al. 1985) was provided by Dr. Rogler. Probes J19.4 (D11S17; Housman et al. 1985) and ES1–2 (D11S9; Gusella et al. 1980) were obtained from D. S. Gerhard. The 0.5-kb *Hinc*II/*Sac*I fragment of pRS1.2 (Watkins et al. 1987) was used as a probe for FSHB. Plasmid p32–1 (D11S16; Feder et al. 1985) was obtained from the ATCC, Rockville, MD.

Isolation of Random DNA Probes

The complete *Hind*III-digest Charon 21A library of flow-sorted chromosomes 11 of the National Gene Library Project (LL11NS01) was used as a source for random clones. Two hundred clones were isolated that hybridized with labeled total human DNA (Gusella et al. 1980), and phage DNA was prepared according to the method of Helms et al. (1985). Inserts were labeled by hexanucleotide priming (Feinberg and Vogelstein 1984) and were preannealed with an excess of sheared placental DNA (Sealey et al. 1985) before being hybridized against hybrid cell mapping panels. Of those inserts giving adequate signal in the first round of screening, 10 were absent from the N.W. deletion hybrid but were present in two cell lines containing normal chromosomes 11. Phage inserts were subcloned into pUC19 to facilitate the search for unique fragments which were then used to verify the chromosome assignment with a larger panel of hybrid cells that had chromosome 11 as the sole shared human chromosome.

The *Eag*I end-fragment library was prepared in EMBL6 phage (Frischauf et al. 1987) from DNA of a human-hamster somatic cell hybrid line which contains human chromosomes 11 and X in nearly all cells, plus chromosomes 6 and 12 in a fraction of cells. Three aliquots of 5 μg DNA were digested with *Eag*I and one of the enzymes *Bam*HI, *Bgl*II or *Bcl*I, which give *Bam*HI-compatible ends. After phosphatase treatment, phenol extraction and dialysis, 0.7 μg of insert DNA were ligated with 1 μg of EMBL6 DNA, which had been

cut with the enzymes *Not*I, *Bam*HI, and *Eco*RI. In vitro packaging with Gigapack Plus (Stratagene, La Jolla) resulted in a library of 20,000 independent clones on *E. coli* strain P2392. Aliquots of the library were plated at low density and were screened with radiolabeled human DNA. Positive clones were rescreened for the absence of hamster repetitive elements, and phage DNA was prepared. Inserts were released by *Sal*I cleavage and were isolated from low-melting-point agarose gels. Chromosomal mapping and subsequent subcloning were done as described for Charon 21A clones.

DNA Extraction

Cultured cells were washed twice in ice-cold PBS and were resuspended in lysis buffer (70 mM NaCl; 10 mM Tris-HCl, pH8; 20 mM Na_2EDTA). After addition of SDS to 0.2% and proteinase K (100 μg/ml), the mixture was incubated at 50°C overnight. Nucleic acids were purified by repeated phenol and chloroform extractions followed by isopropanol precipitation. DNA threads were spooled out, washed in ethanol, and resuspended in TE buffer at 0.2–0.6 mg/ml. DNA from peripheral blood leukocytes was isolated according to a method described by Kunkel et al. (1982).

Preparation of Gene Dosage Blots

DNA was digested at 0.1 μg/μl with *Eco*RI (10 U/μg) for at least 4 h. DNA concentrations of each sample were measured spectrofluorometrically (Brunk et al. 1979) after completion of the digest. Aliquots of 3.5 μg of each DNA sample were loaded on a 0.7% agarose gel and were separated overnight at 45 V. Equal loading was again checked by comparison of ethidium bromide staining, and DNA was transferred to GeneScreen membranes (New England Nuclear, Boston) by alkaline blotting with 0.5 N NaOH, 1.5 M NaCl. Subsequently, filters were neutralized in 50 mM sodium phosphate pH 7.2 (1 M sodium phosphate is 134 g $Na_2HPO_4 \cdot 7\ H_2O$ and 4 ml phosphoric acid/liter), and DNA was fixed by baking and UV cross-linking (Church and Gilbert 1984).

Hybridization and Washing Conditions

Filters were prehybridized in 1 M NaCl, 50 mM sodium phosphate pH 7.2, 50 mM Tris-HCl pH 7.5, 1 mM Na_2EDTA, 1% SDS, 50 μg salmon sperm DNA/ml at 65°C for 1 h. DNA probes labeled by hexamer priming (Feinberg and Vogelstein 1984) were added, and hybridization was carried out overnight. After washing in 40 mM sodium phosphate pH 7.2, 1% SDS at 65°C for at least 1 h with three changes of washing solution, the filters were exposed to Kodak XAR-5 film with an intensifying screen for 12–36 h. Before reprobing was undertaken, the filters were stripped by incubating in 0.1 × TE, 0.1% SDS at 75–80°C for 30 min.

Gene Dosage Analysis

Throughout the study, DNA samples from several different lymphoblastoid cell lines of normal individuals were used as controls on every filter. Hybridization signals for each probe were evaluated independently by two investigators. After visual comparison of signal strength, the relative band intensities were measured with an LKB laser scanning densitometer. Every filter was repeatedly hybridized with probes from within and outside the deletions to obtain reference signals. On several gels additional lanes were loaded with 50% of the normal amount of control DNA to provide additional standards for 2:1 dosage.

Results

The Probe Bank of the WAGR Deletion Region

Ten chromosome 11 probes that mapped within the N.W. WAGR deletion were derived by screening 200 random clones with human inserts from the LL11NS01 flow-sorted chromosome 11 library. Chromosome 11 assignment of the probes was established by hybridization with a DNA mapping panel of seven independent hybrid clones that had chromosome 11 as their only shared human chromosome (Bruns et al. 1979; Davis et al. 1986). Fifteen of the WAGR deletion clones used in the present study had been generated by prior analysis of 300 human clones from the LL11NS01 library (Bruns et al. 1987).

To increase the probability of isolating probes in the vicinity of genes, an *Eag*I end-fragment library was constructed in the vector EMBL6 by using DNA from a human-hamster hybrid that contained mainly human chromosomes 11 and X. The use of the enzyme *Eag*I for construction of the library should bias the representation in favor of CpG-rich islands, which are located at the 5′ end of a number of expressed sequences (Brown and Bird 1986). Of 200 human DNA-containing phage inserts screened, four could be assigned to the chromosome 11 deletion region of N.W.

Of the 29 probes deleted from one chromosome 11 homologue of N.W. isolated by screening random clones from the flow-sorted and hybrid cell chromosome 11 libraries, 23 could be sublocalized to a smaller deletion found in an individual with Wilms tumor, aniridia, and mental retardation (M.J.; table 1). Two additional probes

(J77-PH and J77–2.3X) that map within the N.W. deletion were derived from cloning an AGR deletion breakpoint (Gessler and Bruns 1988).

The CAT Locus and Proximal Probes

To subdivide and further characterize the area covered by the N.W. and M.J. deletions, DNAs from cell lines or from leukocytes of a number of individuals with either the WAGR syndrome or a related phenotype were analyzed. Cytogenetic deletions or other rearrangements had been described for all the individuals (table 1). To detect these heterozygous deletions, gene dosage blots were prepared and were hybridized with the probe bank described above, together with suitable controls.

The catalase gene had previously been used as a marker for chromosome 11p13 and was shown to be centromeric to the M.J. deletion but within the larger N.W. deletion (Glaser et al. 1986). Enzyme analysis had already suggested a heterozygous deletion of the catalase gene in GM5296, GM5518, and GM6803 (Institute of Medical Research Catalogue, 1986 ed.). These findings are supported by the results of gene dosage analysis with the catalase cDNA probe. All cell lines analyzed in figure 1*A* shows a diminished hybridization signal with this probe when compared with control DNA. Densitometric scanning revealed a reduction in signal intensity of about 50%, suggesting the loss of one copy of the gene in all cell lines. Rehybridization of the same filter with a non–chromosome 11 probe (N76) confirmed equal loading of the lanes (fig. 1*B*). With the exception of M.J. and BC, all the cytogenetically described deletions, as well as that in GM5296, included the catalase gene (fig. 2). This was also seen with probe 277.

The centromeric part of the N.W. deletion that is not deleted in M.J. could be split into four intervals. The mapping and cloning of the proximal breakpoint in GM7736 (Gessler and Bruns 1988) allowed the first subdivision with probes 277, 575, J77-PH, and CAT located telomeric to the breakpoint (fig. 2). This interval was further broken up by the proximal breakpoint in GM3808, as only probes 575 and J77-PH were present on both chromosome 11 homologues in this cell line. The interval between the GM7736 and N.W. breakpoints was finally split by the proximal end of the GM8785 deletion, with probe 508 being the most proximal marker in the N.W. deletion and probe 540 being located closer to the GM7736 breakpoint.

The positioning of these probes on the proximal side of the M.J. deletion was confirmed by dosage analysis of GM3118. The deletion in this cell line partially overlaps the M.J. deletion but extends even more proximal than the N.W. breakpoint and includes the marker D11S9 (Glaser et al. 1986). All of the probes defining

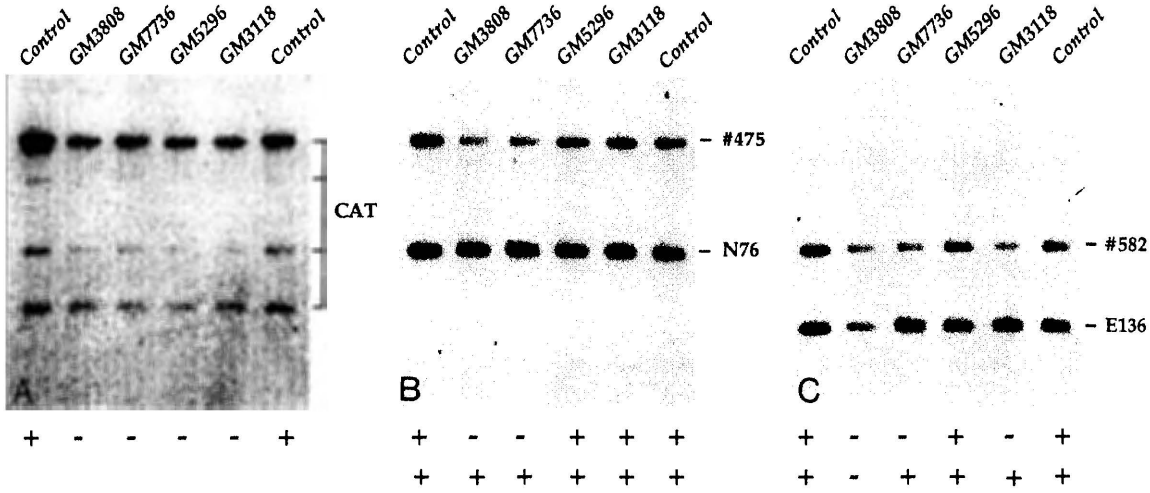

Figure 1 Gene dosage analysis with WAGR deletion probes. A Southern filter prepared with 3.5 μg DNA from different cell lines in each lane was sequentially hybridized with the catalase cDNA probe (*A*), probes 475 and N76 (*B*), and probes 582 and E136 (*C*). Probe N76, which is not derived from chromosome 11, gave an approximately 1:1 ratio of signal strength between test sample and control DNA for all cell lines analyzed. Symbols below the autoradiograms are defined as follows: + = Both alleles present in that particular cell line; - = reduction to a 0.5:1 ratio in signal intensity, indicative of a heterozygous deletion with one allele remaining.

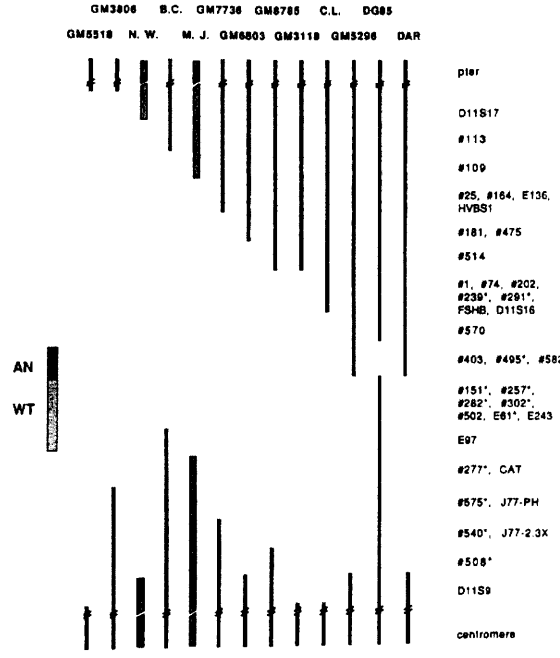

Figure 2 Deletion map of the WAGR region. Probes are listed in a linear order from the most telomeric to D11S9 on the centromeric side. The bars indicate the presence of the respective set of probes. Empty spaces delineate areas of heterozygous deletion. Conserved probes that hybridize to rodent DNA at high stringency are marked by an asterisk (*).

these proximal intervals were heterozygously deleted in GM3118, as expected.

The M.J. deletion could be shown to be completely contained within the larger N.W. deletion. Probes 109 and 113 are present on both homologues in GM3118, as well as in M.J., but are absent from one N.W. homologue. They define, therefore, a new interval distal to the M.J. deletion but within the N.W. deletion (fig. 2). This interval is split by the telomeric breakpoint of the BC deletion which falls between these probes. Only two of the deletions analyzed (GM3808 and GM5518) extended more telomeric on 11p as judged by gene dosage analysis for the D11S17 marker which had been shown to be outside and distal to the N.W. deletion (Glaser et al. 1986).

Definition of Intervals within the M.J. Deletion

The breakpoints of nine cytogenetically defined chromosomal rearrangements could be localized within the M.J. deletion. The mapping and cloning of the GM7736 deletion breakpoints, defined by probe 164, has been described elsewhere (Gessler and Bruns 1988). Three additional probes—25, E136, and HVBS1 (figs. 1C, 2)—are not deleted in GM7736 and therefore map distal to probe 164. All other probes assigned to the M.J. deletion region are absent from one chromosome 11 homologue of GM7736 (figs. 1*b*, 1*c*, 3).

The next proximal interval is defined by GM6803, where two additional probes (181 and 475) did not show reduced dosage. The cell lines GM3118 and GM8785 mark the only instance where two deletion breakpoints within the region defined by the M.J. deletion have not yet been resolved. Probes 181, 475, and 514 are not included in both deletions (figs. 1*B*, 2). As probe 514 was absent from one chromosome 11 homologue in GM6803, the breakpoints in GM3118 and GM8785 can be placed proximal to the GM6803 deletion boundary.

Seventeen of our probes then remained localized to the M.J. deletion but were absent in all the cytogenetically described deletions initially tested. A further subdivision was possible with GM5296. This cell line was described as an insertion of 10q21-q24 into 11p13. The reduced red blood cell catalase activity in the individual from whom the cell line was derived suggested a loss

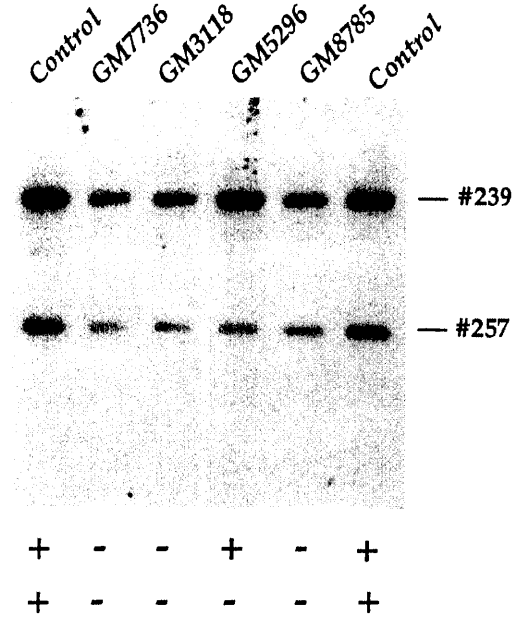

Figure 3 Gene dosage analysis with probes from the proximal part of the M.J. deletion region. Probe 257 is heterozygously deleted in all cell lines (−). For probe 239, three cell lines show deletion of one allele.

of material from chromosome 11. Furthermore all of the probes mapping to the N.W. deletion region proximal to the M.J. breakpoint showed reduced dosage in this cell line, indicating a substantial deletion. The distal end of this deletion allowed us to subdivide the large interval between the GM3118/GM8785 and M.J. breakpoints. Eight of the 17 probes assigned to this area were included in the GM5296 deletion (figs. 1–3) and are therefore located on the centromeric side of this interval. The interval could be further subdivided when leukocyte DNA from patient C.L. was analyzed. Five probes, together with the markers FSHB and D11S16, were present on both chromosome 11 homologues in this individual, whereas probes 403, 495, 570, and 582 and a number of centromeric probes were heterozygously deleted (figs. 2, 4*a*). The distal boundaries of the GM5296 and C.L. deletions, therefore, divide the critical region of overlap of the WAGR deletions into three intervals. A fourth interval on the proximal side of the overlap region, defined by probe E97, was identified by analysis of leukocyte DNA from BC, a male child with genitourinary anomalies, aniridia, and a 11p14-p13 deletion (fig. 4*b*). Seven of the eight probes between the GM5296 and M.J. breakpoints were heterozygously deleted, as well as all telomeric probes tested (fig. 2).

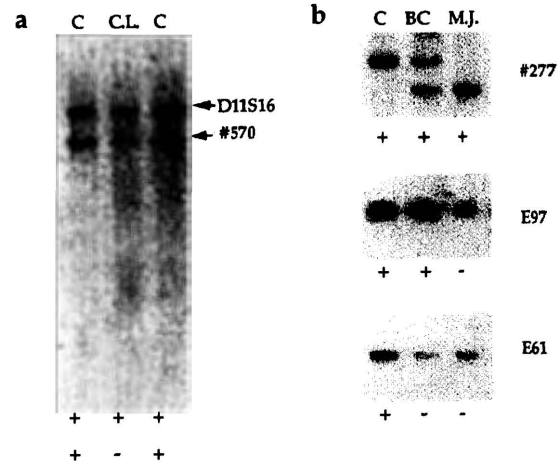

Figure 4 Gene dosage analysis of leukocyte DNA from patients C.L. (*a*) and BC (*b*). Probes D11S16 and 570 were used for C.L. DNA with control DNA (C) run on both sides. Control samples for the analysis of BC. DNA were normal lymphoblastoid DNA (C) and DNA from a fibroblast cell line of M.J. in which a deletion of probes E97 and E61 from one chromosome 11 homologue had been shown by somatic cell hybrid analysis. Probe 277 shows an RFLP with the enzyme *Eco*RI. The presence of both or only one allele for each probe is indicated by (+) and (−), respectively.

Chromosomal Deletions Defining Probes for the Wilms Tumor and Aniridia Genes

In one kindred with heritable isolated aniridia, the defect was associated with the chromosome translocation t(11;22) (p13;q12.2) (Moore et al. 1986). As reported by Davis et al. (1988), this translocation is associated with a cytogenetically undetectable deletion of sequences from chromosome 11. Analysis of a cell line from one individual of this family (D.G.85) revealed that probes 403, 495, and 582 are heterozygously deleted (figs. 2, 5). Earlier analysis of another chromosome 11 translocation associated with familial aniridia had localized the FSHB gene distal to the breakpoint and the presumptive gene (Glaser et al. 1986). This would then put probes 403, 495, and 582 centromeric to FSHB. The same order was derived from analysis of the C.L. deletion. Pulsed-field gel analysis with probes surrounding FSHB (Gessler and Bruns, submitted) has shown that probe 570 must be located telomeric to those deleted in D.G.85, thereby establishing an additional interval defined by probe 570 between the D.G.85 deletion and the distal breakpoint in C.L.

The chromosomal region for the Wilms tumor gene was defined using the hybrid cell line DAR which contains the deleted chromosome 11 of an individual with Wilms tumor but not aniridia (Turleau et al. 1984; Couillin et al., submitted). All of our probes mapping proximal to the D.G.85 deletion were absent in DAR. Eight probes, five of which detect conserved sequences in hamster or mouse DNA, are deleted both in DAR and in the M.J. hybrid cell line. As both individuals had Wilms tumor, all probes from the overlap region of the M.J. and DAR deletions are potential candidate clones to define the Wilms tumor locus.

Discussion

Mapping of random phage clones against hybrid panels containing WAGR deletion chromosomes has allowed us to develop a large probe bank for this region. Twenty-nine new probes covering the area from proximal 11p14 to distal 11p12 have been isolated in this way. Twenty-three of these could be sublocalized to a smaller deletion which does not include the catalase gene.

Eight cell lines with cytogenetically detected chromosome 11p rearrangements, most of them deletions, together with three hybrid cell lines and two leukocyte DNA samples from individuals with WAGR-related de-

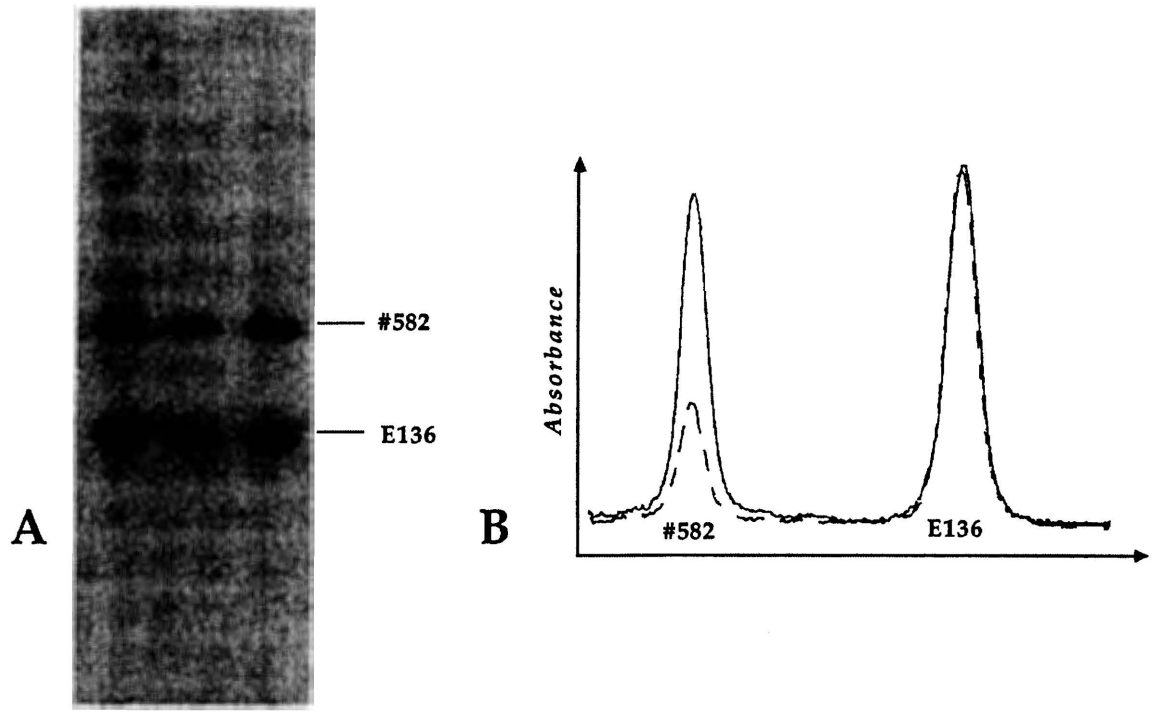

Figure 5 Gene dosage analysis of D.G. 85. *A*, *Eco*RI-digested DNA from D.G. 85 (middle lane) and from two control samples was analyzed with probes 582 and E136. *B*, Densitometric scanning of the autoradiogram revealed a signal intensity ratio of 0.43:1 for probe 582 when D.G. 85 (dashed line) was compared with control lanes (solid line).

letions, were studied by gene dosage analysis. Every cell line showed reduced dosage, indicative of a heterozygous deletion, for at least some of the probes tested. A different subset of the probe bank, however, was affected in each cell line. These partially overlapping deletions allowed us to subdivide the WAGR region of chromosome 11 into 16 intervals, extending from band p14 to band p12. Nearly all of the breakpoints were found to be staggered and set apart from each other by at least one probe. This observation argues against the involvement of a major recombination hot spot in the induction of these rearrangements.

In related studies, Porteous et al. (1987) and Bickmore et al. (1988) have divided the region between mid-11p14 and proximal 11p12 into seven intervals, two of which fall between FSHB and CAT. The subdivision of the WAGR region into 16 intervals in the present study, including five subregions in the overlap area necessary for the pathognomonic features of the syndrome, provides a framework map for the molecular, physical, and genetic analysis of this region. Although specific phenotypes have not yet been associated with deletion subregions beyond the critical region, definition of six intervals above and five intervals below the overlap area may permit identification of subtle phenotypic features in individual patients.

The relative extent of the deletions as analyzed by our dosage studies was in agreement with the cytogenetic description in most of the cases. In GM5296, which contains a chromosomal insertion into 11p13, a cytogenetically undetected deletion had been postulated from the analysis of catalase activity. This deletion could be verified by gene dosage analysis with a catalase cDNA clone. The absence of a number of additional probes from one chromosome 11 homologue of this cell line even suggests that this deletion is of considerable size. Probes close to the breakpoints of complex rearrangements, such as that in GM5296, offer a direct route to identify their exact molecular composition by high-resolution in situ hybridization.

A smaller deletion, including only three of the probes, was found in the cell line of an individual with t(11;22)

(p12;q12.2), associated with heritable aniridia. A similar observation has recently been published by Davis et al. (1988). These probes are important markers for localizing the aniridia gene, which should be within the deletion or very close to the chromosome breakpoints. Determination of the relationship between the breakpoints of this deletion and that of a second translocation, also associated with familial aniridia (Simola et al. 1983), may lead to the identification of the gene.

The deletion region common to the M.J. and DAR hybrid cell lines provides the smallest region of overlap for the Wilms tumor locus. Eight of our probes map to this interval, and five of these eight detect conserved sequences in hamster or mouse DNA. They may, therefore, contain parts of coding sequences. The use of these probes in analysis of DNAs from Wilms tumors may pinpoint this gene. Probe E97 was not included in the deletion found in BC, which restricts the interval for the gene causing anomalies of the genitourinary tract to that between the BC and DAR breakpoints. This does not necessarily exclude probe E97 from the Wilms tumor region, although Wilms tumor and genitourinary anomalies have been postulated to result from different events at the same locus (Porteous et al. 1987). Although several deletions with centromeric boundaries proximal to CAT have been described in females (Glaser et al. 1986; Compton et al. 1987; Porteous et al. 1987), only one, the RIST deletion, has been reported in a male (Davis et al. 1988). As urinary-tract anomalies are often not conspicuous in females, the BC deletion defines for the first time a proximal limit for the GU subregion of WAGR deletions.

Evidence for the localization of the gene causing mental retardation in WAGR patients may come from analysis of the deletion in C.L., who had aniridia and Wilms tumor together with a cytogenetic deletion of 11p13, but who showed average intelligence (Russell and Weisskopf 1986). The deletion in this case extends more proximal than do those in M.J., GM7736, and GM8785, all of which are associated with mental retardation, but it does not extend far on the telomeric side. Similarly, the DAR deletion, which also is extensive on the centromeric side, was not associated with mental retardation (Turleau et al. 1984). By contrast, the deletions in the GM8785 and GM6803 cell lines were reported to be associated with developmental delay in a 5-mo-old child and with frank mental retardation, respectively. We suggest, therefore, that the locus for mental retardation may be between the distal breakpoint of the C.L. deletion and the proximal boundaries of the GM8785 and GM6803 deletions. Definition of the relationship between telomeric extent and the presence or absence of mental retardation for additional deletions will be necessary to evaluate this model.

In addition to defining the WAGR gene loci, this high-resolution map derived from our gene dosage studies will also serve as an invaluable tool for the analysis of any new WAGR-related deletion. All additional breakpoints can now be mapped more precisely and can be localized relative to breakpoints described in the present study. A number of these breakpoints will be close enough to our probes to permit molecular cloning, thereby providing insight into the mechanisms inducing these rearrangements. The high density of probes will also make it possible to develop a continuous long-range restriction map of this region. Given an estimated 10 Mbp size for the N.W. deletion, the average distance between probes will be on the order of only a few hundred kilobase pairs.

Acknowledgments

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References

Bickmore, W., S. Christie, V. van Heyningen, N.D. Hastie, and D. J. Porteous. 1988. Hitch-hiking from HRAS1 to the WAGR locus with CMGT markers. Nucleic Acids Res. 16:51–60.

Brown, W. R. A., and A. P. Bird. 1986. Long-range restriction mapping of mammalian genomic DNA. Nature 322: 477–481.

Brunk, C., K. Jones, and T. James. 1979. Assay for nanogram quantities of DNA in cellular homogenates. Anal. Biochem. 92:497–500.

Bruns, G. A. P., S. D. Barnes, M. Gessler, J. B. Brennick, and M. J. Weiner. 1987. DNA probes for chromosome 11 and the WAGR deletion. Cytogenet. Cell Genet. 46:588.

Bruns, G. A. P., T. Glaser, J. F. Gusella, D. E. Housman, and S. H. Orkin. 1984. Chromosome 11 probes identified with a catalase oligonucleotide. Am. J. Hum. Genet. 36:25S.

Bruns, G. A. P., B. J. Mintz, A. C. Leary, V. M. Regina, and P. S. Gerald. 1979. Human lysosomal genes: arylsulfatase A and beta-galactosidase. Biochem. Genet. 17:1031–1059.

Church, G. M., and W. Gilbert. 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA 81:1991–1995.

Compton, D. A., M. M. Weil, L.-Y. Chao, W. H. Lewis, C. Jones, V. M. Riccardi, L. C. Strong, and G. F. Saunders. 1987. Physical analysis of chromosomal band 11p13 using random probes and field inversion gel electrophoresis. Am. J. Hum. Genet. 41:A162.

Couillin, P., M. Azoulay, I. Henry, N. Ravise, M. C. Grisard, C. Jeanpierre, F. Barichard, P. Metezeau, J. J. Candelier, W. Lewis, V. van Heyningen, and C. Junien. Characterization of a panel of somatic cell hybrids for subregional mapping along 11p and within band 11p13: subdivision of the WAGR complex region. (submitted).

Davis, A. E. III, A. S. Whitehead, R. A. Harrison, A. Dauphinais, G. A. P. Bruns, M. Cicardi, and F. S. Rosen. 1986. Human inhibitor for the first component of complement, C1: characterization of cDNA clones and localization of the gene on chromosome 11. Proc. Natl. Acad. Sci. USA 83:3161–3165.

Davis, L. M., R. Stallard, G. H. Thomas, P. Couillin, C. Junien, N. J. Nowak, and T. B. Shows. 1988. Two anonymous DNA segments distinguish the Wilms' tumor and aniridia loci. Science 241:840–842.

de Martinville, B., and U. Francke. 1983. The c-Ha-ras1, insulin and beta-globin loci map outside the deletion associated with aniridia–Wilms' tumor. Nature 305:641–643.

Douglas, E. C., J. A. Wilimas, A. A. Green, and A. T. Look. 1985. Abnormalities of chromosome 1 and 11 in Wilms' tumor. Cancer Genet. Cytogenet. 14:331–338.

Feder, J., L. Yen, E. Wijsman, L. Wang, L. Wilkins, J. Schroder, N. Spurr, H. Cann, M. Blumenberg, and L. L. Cavalli-Sforza. 1985. A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. Am. J. Hum. Genet. 37:635–649.

Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabeling of DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 137:266.

Ferrell, R. E., and V. M. Riccardi. 1981. Catalase levels in ptients with aniridia and/or Wilms' tumor: utility and limitations. Cytogenet. Cell Genet. 31:120–123.

Francke, U., L. B. Holmes, L. Atkins, and V. M. Riccardi. 1979. Aniridia-Wilms' tumor association: evidence for specific deletion of 11p13. Cytogenet. Cell Genet. 24: 185–192.

Frischauf, A. M., N. Murray, and H. Lehrach. 1987. Lambda phage vectors—EMBL series. Methods Enzymol. 153: 103–116.

Gessler, M., and G. A. P. Bruns. 1988. Molecular mapping and cloning of the breakpoints of a chromosome 11p14.1-p13 deletion associated with the AGR syndrome. Genomics 3:117–123.

_____. A physical map around the WAGR complex on the short arm of chromosome 11. (submitted).

Glaser, T., W. H. Lewis, G. A. P. Bruns, P. C. Watkins, C. E. Rogler, T. B. Shows, V. E. Powers, H. F. Willard, J. M. Goguen, K. O. J. Simola, and D. E. Housman. 1986. The beta-subunit of follicle-stimulating hormone is deleted in patients with aniridia and Wilms' tumor, allowing a further definition of the WAGR locus. Nature 321:882–887.

Gusella, J. F., C. Keys, A. Vasaranyi-Breiner, F.-T. Kao, C. Jones, T. T. Puck, and D. Housman. 1980. Isolation and localization of DNA segments from specific human chromosomes. Proc. Natl. Acad. Sci. USA 77:2829–2833.

Helms, C., M. J. Graham, J. E. Dutchik, and M. V. Olson. 1985. A new method for purifying lambda DNA from phage lysates. DNA 4:39–49.

Housman, D. E., D. S. Gerhard, T. Glaser, and C. Jones. 1985. Mapping of chromosome 11p by linkage and somatic cell genetic techniques: a comparison of relative map order derived by each method. Cytogenet. Cell Genet. 40:655.

Junien, C., C. Turleau, J. de Grouchy, R. Said, M.-O. Rethore, R. Tenconi, and J. L. Dufier. 1980. Regional assignment of catalase (CAT) gene to band 11p13: association with the aniridia–Wilms' tumor–gonadoblastoma (WAGR) complex. Ann. Genet. 23:165–168.

Junien, C., C. Turleau, G. M. Lenoir, T. Philip, R. Said, S. Despoisse, C. Laurent, M. O. Rethore, J. C. Kaplan, and J. de Grouchy. 1983. Catalase determination in various etiologic forms of Wilms' tumor and gonadoblastoma. Cancer Genet. Cytogenet. 7:51–57.

Kaneko, Y., M. C. Egues, and J. D. Rowley. 1981. Interstitial deletion of the short arm of chromosome 11 limited to Wilms' tumor cells in a patient without aniridia. Cancer Res. 41:4577–4578.

Kunkel, L. M., U. Tantravahi, M. Eisenhard, and S. A. Latt. 1982. Regional localization on the human X of DNA segments cloned from flow sorted chromosomes. Nucleic Acids Res. 10:1557–1578.

Moore, J. W., S. Hyman, S. E. Antonarakis, E. H. Mules, and G. H. Thomas. 1986. Familial isolated aniridia associated with a translocation involving chromosomes 11 and 22 [t(11;22) (p13;q12.2)]. Hum. Genet. 72:297–302.

Porteous, D. J., W. Bickmore, S. Christie, P. A. Boyd, G. Cranston, J. M. Fletcher, J. R. Gosden, D. Rout, A. Seawright, K. O. J. Simola, V. van Heyningen, and N. D. Hastie. 1987. HRAS1-selected chromosome transfer generates markers that colocalize aniridia- and genitourinary dysplasia-associated translocation breakpoints and the Wilms tumor gene within band 11p13. Proc. Natl. Acad. Sci. USA 84:5355–5359.

Riccardi, V. M., H. M. Hittner, U. Francke. J. J. Yunis, D. Ledbetter, and W. Borges. 1980. The aniridia–Wilms' tumor association: the critical role of chromosome band 11p13. Cancer Genet. Cytogenet. 2:131–137.

Rogler, C. E., M. Sherman, C. Y. Su, D. A. Shafritz, J. Summers, T. B. Shows, A. Henderson, and M. Kew. 1985. Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. Science 230: 319–322.

Russell, L. J., and B. Weisskopf. 1986. Cognition in aniridia–Wilms tumor association: analysis of karyotype association. Am. J. Hum. Genet. 39:A78.

Sealey, P. G., P. A. Wittaker, and E. M. Southern. 1985. Removal of repeated sequences from hybridization probes. Nucleic Acids Res. 13:1905–1923.

Shannon, R. S., J. R. Mann, E. Harper, D. G. Harnden, J. E. N. Morten, and A. Herbert. 1982. Wilms' tumor and aniridia: clinical and cytogenetic features. Arch. Dis. Child. 57:685–690.

Simola, K. O. J., S. Knuutila, I. Kaitila, A. Pakola, and P. Pohja. 1983. Familial aniridia and translocation t(4;11)(q22;p13) without Wilms' tumor. Hum. Genet. 63:158–161.

Turleau, C., J. de Grouchy, C. Nihoul-Fekete, J. L. Dufier, F. Chavin-Colin, and C. Junien. 1984. Del11p13/nephroblastoma without aniridia. Hum. Genet. 67:455–456.

Watkins, P. C., R. Eddy, A. K. Beck, V. Vellucci, B. Leverone, R. E. Tanzi, J. F. Gusella, and T. B. Shows. 1987. DNA sequence and regional assignment of the follicle-stimulating hormone beta-subunit gene to the short arm of human chromosome 11. DNA 6:205–212.