

Pericentric Intrachromosomal Insertion Responsible for Recurrence of del(11)(p13p14) in a Family

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The combined use of qualitative and quantitative analysis of 11p13 polymorphic markers together with chromosomal in situ suppression hybridization (CISS) with biotin labeled probes mapping to 11p allowed us to characterize a complex rearrangement segregating in a family. We detected a pericentric intrachromosomal insertion responsible for recurrence of del(11)(p13p14) in the family: an insertion of band 11p13-p14 carrying the genes for predisposition to Wilms' tumor, *WT1*, and for aniridia, *AN2*, into the long arm of chromosome 11 in 11q13-q14. Asymptomatic balanced carriers were observed over three generations. Classical cytogenetics had failed to detect this anomaly in the balanced carriers, who were first considered to be somatic mosaics for del(11)(p13). Two of these women gave birth to children carrying a deleted chromosome 11, most likely resulting from the loss of the 11p13 band inserted in 11q. Although in both cases the deletion encompassed exactly the same maternally inherited markers, there was a wide variation in clinical expression. One child, with the karyotype 46,XY,del(11)(p13p14), presented the full-blown WAGR syndrome with aniridia, mental retardation, Wilms' tumor, and pseudohermaphroditism, but also had proteinuria and glomerular sclerosis reminiscent of Drash syndrome. In contrast, the other one, a girl with the karyotype 46,XX,del(11)(p13), only had aniridia. Although a specific set of mutational sites has been observed in Drash patients, these findings suggest that the loss of one copy of the *WT1* gene can result in similar genital and kidney abnormalities. *Genes Chrom Cancer* 7:57-62 (1993). © 1993 Wiley-Liss, Inc.

The WAGR syndrome is a contiguous gene syndrome associated with an interstitial deletion of band 11p13; it consists of Wilms' tumor (WT), aniridia (A), genitourinary abnormalities (G), and mental retardation (R) (Francke et al., 1979). Band 11p13 harbors a number of genes involved in the differentiation and growth control of the eye, kidney, and urogenital tract. Two candidate genes have been isolated recently. The first one, *WT1*, is a zinc finger gene with a proline/glutamine-rich region in the N-terminal portion. It was isolated by positional cloning as a candidate Wilms' tumor predisposition gene and is now widely accepted as a tumor suppressor gene (Call et al., 1990; Gessler et al., 1990; Rose et al., 1990). Tumor specific and constitutional mutations have been described (Haber et al., 1990; Pelletier et al., 1991b). As shown by point mutations in *WT1* in patients with the triad glomerulopathy, Wilms' tumor, and ambiguous genitalia known as the Drash syndrome (Pelletier et al., 1991a), this gene participates in kidney and genitourinary development (Pritchard-Jones et al., 1990; van Heyningen et al., 1990). For the second gene, *AN2*, candidate sequences have also been cloned (Mannens et al., 1991; Ton et al., 1992; van Heyningen et al., 1992). Among these, the human homolog of the *Pax-6* gene, which car-

ries the murine *small eye* mutations, corresponds to a transcription factor with a paired-box domain, a homeodomain, and a serine/threonine-rich carboxy-terminal domain. Different laboratories have constructed maps for 11p13 and numerous markers are available to study this part of chromosome 11 (Junien and van Heyningen, 1991).

Using 11p13 markers, we have characterized a rearrangement observed in a family with two once-removed first cousins carrying del(11)(p13) associated with a complete WAGR syndrome in one case and isolated aniridia in the other. The differences in the clinical features for these two cases could be due either to incomplete penetrance of this syndrome, since 68% of the patients with del(11)(p13) and aniridia develop WT, or to a difference in the size of the deletion.

By genotyping, gene dosage analysis, and chromosomal in situ suppression hybridization (CISS) we determined the extent of the deletion in the two del(11)(p13) cases and identified the rearranged chromosome inherited from the transmitting individuals.

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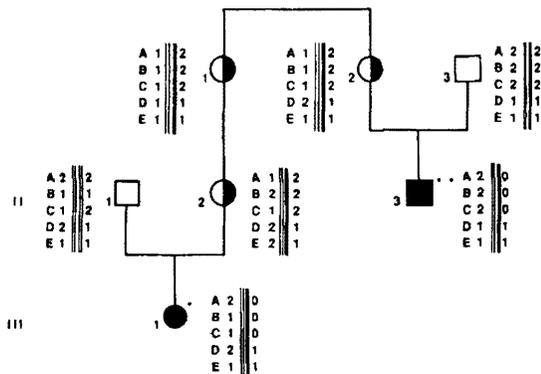


Figure 1. Pedigree and genotypes of the family. Alleles were named 1 and 2 according to decreasing length: 1/0 or 2/0 means that the individual was deleted for one allele. A = *FSHB*; B = *D11S16*; C = *D11S324*; D = *D11S325*; E = *D11S414*. For markers A and C only one of the four possible phases is displayed. * = Patient presenting with $\text{del}(11)(\text{p}13)$ and aniridia. ** = Patient presenting with $\text{del}(11)(\text{p}13)$, aniridia, genitourinary abnormalities, mental retardation, and Wilms' tumor.

The pedigree of the family is shown in Figure 1. Patient II3 had the karyotype 46,XY, $\text{del}(11)(\text{p}13)$. A complete WAGR syndrome was present: aniridia, genitourinary abnormalities with pseudohermaphroditism stage III, and mental retardation. The child had ambiguous external genitalia with a peno-clitoris and a vagina. There was no uterus. The right intraperitoneal gonad was a streak undefined gonad, whereas the left labioscrotal gonad resembled a testis. The child was raised as a girl because of the ambiguous genitalia. The patient developed a Wilms' tumor at age two, had moderate proteinuria starting in the neonatal period without kidney deficiency, and had glomerular sclerosis of the renal parenchyma. Patient III1 presented with aniridia but had no evidence of genitourinary abnormalities, mental retardation, or Wilms' tumor. The karyotype was 46,XX, $\text{del}(11)(\text{p}13)$. No clinical features of the WAGR syndrome were observed in the obligate carriers I1, I2, and II2.

DNA was prepared from EBV-established lymphoblastoid cell lines. For gene copy number analyses the same Southern blots were successively hybridized with an 11p13 probe and with a non-chromosome 11 probe. The intensity of the hybridization signals was measured with a SEBIA densitometer. The value of the 11p13 probe versus non-11 internal control probe was calculated for each independent determination. A ratio of patient versus control close to 1 indicated that there were two copies of the marker in the DNA of the control individual and in the DNA of the patient, while a

ratio close to 0.5 indicated that there were two copies of the marker in the DNA of the control individual and only one copy in the patient's DNA.

The probes used for Southern blot and in situ hybridization are listed in Table 1. Probes were localized by calculating FLpter values or relative to breakpoints (Hoovers et al., 1992). The order of *D11S87*, *WT1*, *D11S324*, *FAT5*, and *ZnFP17* has been determined using molecular techniques (Junien and van Heyningen, 1991; Heding et al., 1992; Ton et al., 1992).

CISS hybridization was performed according to Lichter et al. (1990) but with the modifications described by Hoovers et al. (1992). Briefly, the cosmid probes were ethanol-precipitated in the presence of 50–500-fold concentration of total human DNA. After denaturation of the probe mixture at 70°C, preannealing of repetitive DNA sequences was performed for 1 hour at 37°C. The centromere-specific probe was denatured separately. FITC-conjugated avidin was used for the detection of biotinylated probes. When necessary, the signal was amplified, using biotinylated goat-anti-avidin and a second layer of FITC-conjugated avidin.

For genotyping, DNA from individuals I1, I2, I3, II1, II2, II3, and III1 was digested with the appropriate enzymes and hybridized following Southern blot experiments, using 11p13 markers mapping in the following order: cen-D11S9-*CAT*-*D11S414*/*D11S325*-*WT1*-*AN2*-*D11S323*-*D11S324*-*D11S151*-*D11S16*/*D11S317*-*FSHB*-tel (Junien and van Heyningen, 1991). The genotypes obtained are listed in Table 2. Only informative markers which allowed us to determine the haplotypic phases for 11p13 are represented in Figure 1. None of the patients inherited alleles from their mothers at loci *FSHB* (A), *D11S16* (B), and *D11S324* (C). Patients II3 and III1 inherited the same alleles for *D11S325* (D) and *D11S414* (E) from individual I1. Individuals I1, II1, and III1 inherited the same haplotype (D1-E1) for the D and E loci, showing that the deletion occurred on the same chromosome.

To determine the size of the deletions observed by cytogenetic and genetic analyses in patients II3 and III1, the gene copy number was determined on the DNA of both patients, using probes *D11S317*, *D11S151*, *CAT* (data not shown), *FSHB*, *D11S325*, and *D11S414* (Table 3). *COL1A1* was used as a non-11p internal control. Both patients had only one copy of the sequences corresponding to *FSHB*, *D11S317*, and *D11S151* but displayed two copies of the sequences corresponding to

TABLE 1. Probes: Description and Localization of the Different 11p Probes Used for Chromosome In Situ Suppression Hybridization*

D-Number Genes	Cosmid Probe	FLpter 95% c.i.	Band	Patient Analysis	References
D11S834	ZnFP65	0.148-0.167	p14.3-p15.1	0	Hoovers et al., 1992
D11S780	A403			0	Harrisson-Lavoie et al., 1989
	ZnFP57	0.168-0.187	p14.3-p15.1	0	Hoovers et al., 1992
	C444b	0.195-0.234	p13-p14.3	0	Harrisson-Lavoie et al., 1989
<i>FSHB</i>	C464			0	Harrisson-Lavoie et al., 1989
D11S151	e3012			1	Junien and van Heyningen, 1991
D11S781	ZnFP17	0.215-0.231	p13-p14.1	1	Junien and van Heyningen, 1991
AN2	FAT5		p13	1	Hoovers et al., 1992
DS11S324	p60		p13	1	Gift from V. van Heyningen
<i>WT1</i>	<i>WT1</i>		p13	1	Junien and van Heyningen, 1991
D11S87	CI.1		p13	1	Call et al., 1990
D11S325			p13	0	Junien and van Heyningen, 1991
D11S414			p13	0	Junien and van Heyningen, 1991
D11S782	e31g8	0.259-0.288	p12-p13	0	Gift from P. Little
	ZnFP13	0.262-0.285	p12-p13	0	Hoovers et al., 1992
CAT			p12	0	Junien and van Heyningen, 1991
D11S9				0	Junien and van Heyningen, 1991
	L201	0.297-0.321	p11.2-p12	0	Wadey et al., 1990
D11Z1	pLC11A			0	Waye et al., 1987

*FLpter = relative distance of the probe tested to pter; c.i. = confidence interval; 1 = probe is deleted/inserted; 0 = probe is not deleted/inserted; --- = chromosomal breakpoint known. When no FLpter values are given, the localization is based on cytogenetic banding studies.

TABLE 2. Genotypes of the Different Individuals*

	A <i>FSHB</i> <i>HindIII</i>	B S16 <i>MspI</i>	S151 <i>PstI</i>	C S324 <i>TaqI</i>	S323 <i>HaeIII</i>	D S325 <i>BglIII</i>	S325 <i>HindIII</i>	E S414 <i>TaqI</i>	S414 <i>XmnI</i>	CAT <i>Avall</i>
I1	1/2	1/2	2/2	1/2	2/2	1/1	2/2	1/1	1/1	1/2
I2	1/2	1/2	2/2	1/2	2/2	1/2	2/2	1/1	1/1	1/2
I3	2/2	2/2	2/2	2/2	2/2	1/1	2/2	1/1	1/1	1/2
III1	2/2	1/1	2/2	1/2	1/2	1/2	1/2	1/1	1/1	1/2
II2	1/2	2/2	2/2	1/2	2/2	1/2	2/2	1/2	1/2	1/2
II3	2/0	2/0	2/0	2/0	2/0	1/1	2/2	1/1	1/1	1/2
III1	2/0	1/0	2/0	1/0	1/0	1/2	1/2	1/1	1/1	1/2

**FSHB* (A), S16 = D11S16 (B), S151 = D11S151, S324 = D11S324 (C), S323 = D11S323, S325 = D11S325 (D), S414 = D11S414 (E). Alleles were named 1 and 2 according to decreasing length; 0 = deleted allele.

D11S414, D11S325, and *CAT*. The deletions observed involved the same 11p13 markers from *FSHB*, at least, including D11S151 (Table 3; Fig. 2). With the markers used we were not able to define the distal breakpoints by gene dosage analysis.

Probe *Znfp17* (D11S781) is specific for 11p13 and was used to perform in situ hybridization on

metaphases obtained from the asymptomatic transmitting individuals I1, I2, and II2 and from the patients II3 and III1. The sequence corresponding to *Znfp17* was found to be transferred to the proximal long arm for one of the two homologous chromosomes 11 for individuals I1, I2, and II2 (Fig. 3A). In patients II3 and III1, there was no signal corresponding to *Znfp17* either on 11p13 or on

TABLE 3. Gene Copy Number Determination, Densitometry Data^a

	FSHB Probe				S325 Probe				S414 Probe			
	FSHB	COL1A1	R	P/C	S325	COL1A1	R	P/C	S414	COL1A1	R	P/C
CONTROL	276	164	1.68		893	573	1.56		1,709	1,068	1.60	
III1	192	188	1.02	0.60	605	411	1.47	0.94	1,812	1,135	1.59	0.99
II3	105	94	1.11	0.66	803	441	1.82	1.16	1,823	952	1.91	1.19

^aDensitometry values obtained by scanning the blots shown in Figure 2. R = ratio obtained by dividing the intensity for the 11p13 marker by the intensity of the non-11p internal control (COL1A1); P/C = ratio obtained by dividing the preceding ratio for the patient by that of the control individual; S325 = D11S325; S414 = D11S414.

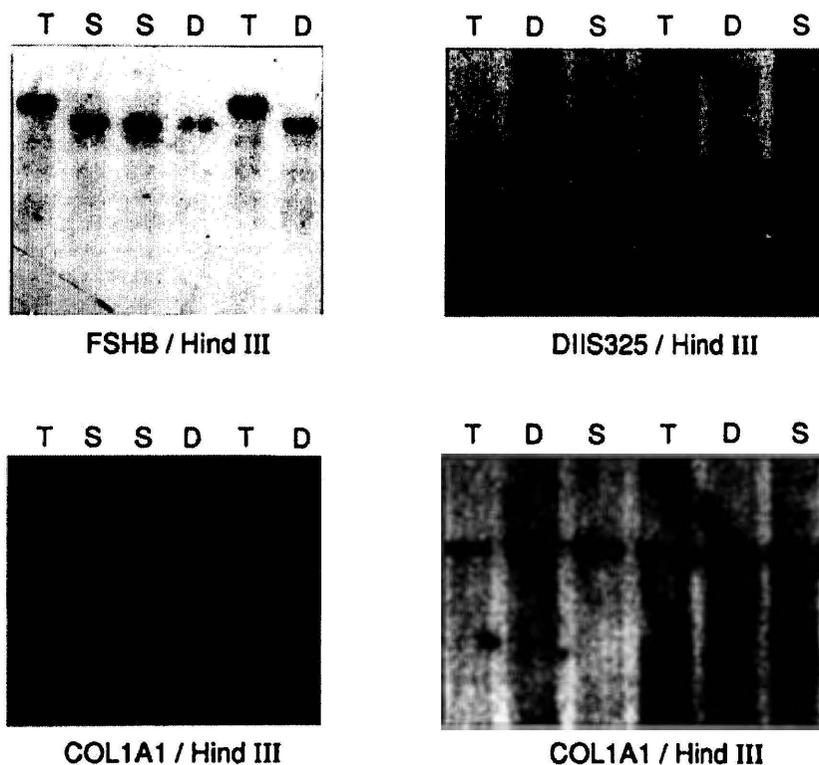


Figure 2. Gene copy number determination for patients II3 and III1. Top: 11p13 probes: FSHB and D11S325. Bottom: The same filters were hybridized with a non-11p probe, COL1A1, used as the internal control. Lanes T = control individuals; lanes D = patient II3; lanes S = patient III1.

11q21 for one of the two homologous chromosomes 11 (Fig. 3B). In order to define more precisely the region rearranged, markers spanning the region between 11p15.1 (D11S834) and the centromere (D11Z1) were also used on metaphases from patient III1. As shown in Table 1, the signals corresponding to the markers D11S781, *FAT5*, D11S324, *WT1*, and D11S87 were translocated on the long arm of chromosome 11 for individuals I1 and I2, and were absent in patients II3 and III1. Normal signal locations were obtained with the other telomeric and centromeric flanking markers.

Using polymorphic markers and chromosomal in situ suppression hybridization (CISS) we have demonstrated that in one pedigree two related cases of (W)A(GR) associated with a deletion of 11p13-p14 resulted from the transmission of a pericentric intrachromosomal insertion of the WAGR region in three members (I1, I2, II2) and probably in the mother or father of I1 and I2. CISS and RFLP analysis enabled us to determine the boundaries of the insertion/deletion. Although complicated alternative pachytene diagrams can be constructed for this type of rearrangement (Madan and

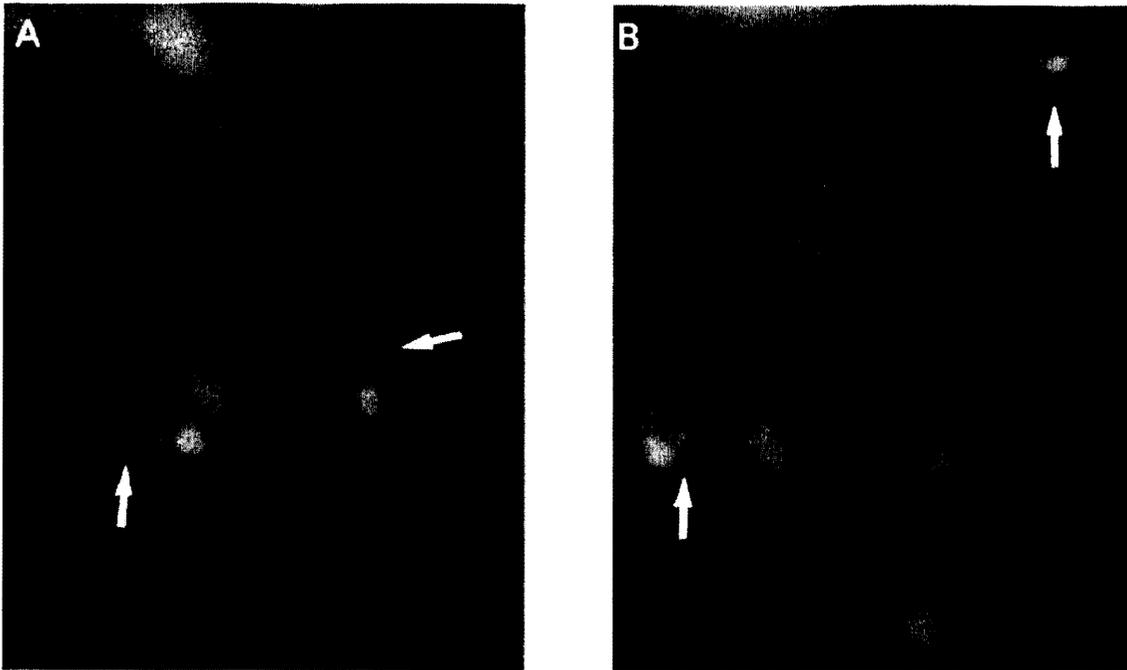


Figure 3. Representative in situ hybridization. **A:** Hybridization of probes D11S781 (cosmid in the WAGR region) and D11Z1 (centromere-specific probe for chromosome 11) to metaphases from individual I2. On the right, the normal chromosome 11 with cosmid signal on band p13-p14.1. On the left, the rearranged chromosome 11 with D11S781 signal on the proximal part of the long arm of chromosome 11, and absence of the signal on 11p13-p14.1. **B:** The same probes, hybridized to metaphase chromosomes from patient II3. On the left, the normal chromosome 11; and on the right, the rearranged chromosome 11 showing deletion of D11S781.

Menko, 1992), in the most simple explanation the deleted chromosome 11 is the result of a homologous pairing that excluded the inserted segment and its homolog on the normal chromosome 11. A single crossover (or any uneven number of crossovers) between these segments will give two recombinant chromosomes: one with a deletion and one with a duplication of the WAGR region. Madan and Menko (1992) reviewed 27 cases of intrachromosomal insertions and gave a general risk of 15% for children to inherit an unbalanced karyotype. In this case the risk might be higher because of the viability of conceptus with either a deletion or a duplication of the WAGR region (Lavedan et al., 1989).

Among the 71 WAGR syndromes reported, 54 (76%) were sporadic cases with de novo rearrangements of 11p13 and 15 (21%) were familial cases. The size of the deletion is variable (Junien and van Heyningen, 1991). One sporadic case of *del(11)(p13)* in the mosaic state (50%) was described by Nakagome and Nagahara (1985). This mosaicism could be the result of an early postzygotic rearrangement. Most of the de novo rearrangements are of paternal origin (Huff et al.,

1990). Among the 15 familial cases described, the nature of the rearrangement was determined in 6. Five resulted from a balanced insertion of band 11p13 into another chromosome (Hittner et al., 1979; Koussef, 1981; Yunis and Ramsay, 1980; Lavedan et al., 1989; Prieur, 1989), one from a ring chromosome inherited from the mother with a mosaic 47,XX,*del(11)(p11p15)*, +*r(11)(p11p15)*/46,XX,*del(11)(p11p15)* (Mannens et al., 1991). They all involved maternal inheritance except one case of insertion (Lavedan et al., 1989). Altogether these observations suggest that the asymptomatic carriers presented with an apparently balanced rearrangement. The deletion could be of paternal or maternal origin without differences in the expression of the syndrome. In the family reported here, both affected individuals with complete and incomplete forms of WAGR had inherited the deleted chromosome, including the gene for both *WT1* and *FAT5*, a candidate sequence for aniridia, via the maternal germline. The incomplete penetrance observed in WAGR patients is thus not correlated with the parental origin of the inherited rearranged chromosome. Together with the demonstration of a biallelic expression of the *WT1* gene

(Zhang and Tycko, 1992), our results do not argue in favor of imprinting as an explanation for the variable expressivity in WAGR cases.

Interestingly, the patient with the more severe WAGR syndrome, including pseudohermaphroditism, had the karyotype 46,XY,del(11)(p13). The less severely affected patient was a girl with exactly the same deletion, 46,XX,del(11)(p13). The observation of proteinuria and glomerular sclerosis in patient II3 is reminiscent of Drash syndrome for which specific point mutations in *WT1* have been described. As proposed by Pelletier and coworkers (1991a, b), these mutations in exons 9 and 8 could have negative dominant effects and be more deleterious than the loss of one *WT1* allele. It is however, interesting that patient II3 with deletion of *WT1* presented features similar to those of the Drash syndrome. Since most cases of Drash syndrome are children with a 46,XY karyotype and ambiguous genitalia, the presence of the Y chromosome and either a mutated or a deleted allele of *WT1* has a more serious effect on external and internal gonad development.

The combined use of polymorphic markers and CISS thus proved to be a powerful tool to characterize a complex rearrangement undetectable by classical cytogenetic methods.

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