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Inherited Interstitial Duplications of Proximal 15q: Genotype-Phenotype Correlations

C. E. Browne,[1] N. R. Dennis,[2] E. Maher,[3] F. L. Long,[1] J. C. Nicholson,[1] J. Sillibourne,[1] and J. C. K. Barber[1]

[1]Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, United Kingdom; [2]Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom; and [3]Division of Medical Genetics, Department of Paediatrics and Child Health, University of Birmingham, Birmingham Women's Hospital, Birmingham, United Kingdom

Summary

We present the cytogenetic, molecular cytogenetic, and molecular genetic results on 20 unrelated patients with an interstitial duplication of the proximal long arm of chromosome 15. Multiple probes showed that the Prader-Willi/Angelman critical region (PWACR) was included in the duplication in 4/20 patients, each ascertained with developmental delay. The duplication was also found in two affected but not in three unaffected sibs of one of these patients. All four probands had inherited their duplication from their mothers, three of whom were also affected. Two of the affected mothers also carried a maternally inherited duplication, whereas the duplication in the unaffected mother and in an unaffected grandmother was paternal in origin, raising the possibility of a parental-origin effect. The PWACR was not duplicated in the remaining 16 patients, of whom 4 were referred with developmental delay. In the 14 families for which parental samples were available, the duplication was inherited with equal frequency from a phenotypically normal parent, mother or father. Comparative genomic hybridization undertaken on two patients suggested that proximal 15q outside the PWACR was the origin of the duplicated material. The use of PWACR probes discriminates between a large group of duplications of no apparent clinical significance and a smaller group, in which a maternally derived PWACR duplication is consistently associated with developmental delay and speech difficulties but not with overt features of either Prader-Willi syndrome or Angelman syndrome.

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Address for correspondence and reprints: Caroline E. Browne, Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, SP2 8BJ, Wilts, United Kingdom. E-mail: wessex.genetics@dial.pipex.com

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Introduction

Structural rearrangements of the 15q11-q13 region, including deletions, translocations, inversions, and supernumerary marker chromosomes, as well as uniparental disomy (UPD) for chromosome 15, have all been reported in patients with Prader-Willi syndrome (PWS) and Angelman syndrome (AS) (Ledbetter et al. 1982; Mattei et al. 1983; Kaplan et al. 1987; Magenis et al. 1987; Butler 1990; Williams et al. 1990; Robinson et al. 1991; Clayton-Smith et al. 1992; Webb et al. 1992). Deletion or disruption of the Prader-Willi/Angelman critical region (PWACR) within 15q12 may result in either PWS or AS when the affected homologue is paternally or maternally inherited, respectively. This parent-of-origin effect has been explained as a consequence of oppositely imprinted genes within this region (Reis et al. 1994; Sutcliffe et al. 1994).

By contrast, cytogenetic duplications of 15q11-q13 have been more frequently reported in phenotypically normal individuals, in patients with developmental delay and/or learning difficulties, and in patients with a range of nonspecific abnormalities, as well as in the occasional patient with PWS or AS (de France et al. 1984; Fuhrmann-Rieger et al. 1984; Pettigrew et al. 1987; Ludowese et al. 1991; Clayton Smith et al. 1993; Bundey et al. 1994; Jalal et al. 1994). In the majority of these cases, the proband inherited the dup(15) from a phenotypically normal parent. Consequently, many of these dup(15)s were interpreted as euchromatic variants without clinical effect. The majority of reported cases had, however, only been investigated by use of conventional cytogenetic techniques.

We present the results of molecular cytogenetic and molecular genetic investigations conducted on 20 patients with a cytogenetic duplication of proximal 15q (fig. 1) and on their families. Our results demonstrate that these apparently identical cytogenetic duplications differ at the molecular level and that, by use of parental-origin studies and molecular investigation with probes from within the PWACR, new genotype-phenotype correlations can be made.

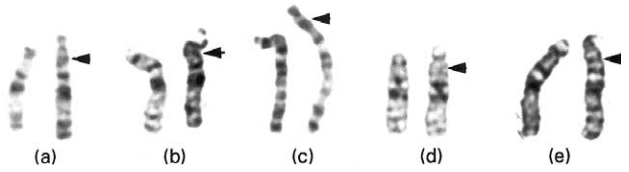

Figure 1 Partial G-banded karyotypes from three patients with a dup(15) including PWACR (*a–c*) and from two patients with a dup(15) excluding PWACR (*d* and *e*). The dup(15) is shown on the right of each pair.

Patients and Methods

Patients

Fifteen of our 20 patients were ascertained at the Wessex Regional Genetics Laboratory during 1983–96, while undergoing routine cytogenetic analysis for a variety of referral reasons (table 1). The remaining five patients were referred to us as known dup(15) carriers. Peripheral blood was obtained from the patients, their parents, and other family members, when available, and was used for both molecular cytogenetic and molecular genetic studies.

Conventional Cytogenetic Studies

Conventional cytogenetic analyses were performed on the patients and on their parents by use of a standard phytohemagglutinin-stimulated lymphocyte-culture method followed by G-banding (GTG) and DA/DAPI staining. C-banding (CBG) was performed on three of our earliest patients (patients 1, 2, and 10). However, this was superseded by in situ hybridization with a chromosome 15 centromere probe for all subsequent patients (see below). Slides for FISH were prepared in the same way but were aged for 24–48 h prior to hybridization.

FISH and Molecular Genetics

FISH studies were performed by use of the standard method described by Pinkel et al. (1988). Hybridization signals from biotin- or digoxigenin (DIG)-labeled probes were detected by use of either single layers of avidin-fluorescein isothiocyanate (for biotin) or an anti-DIG-TRITC. The probes used were pTRA-20 (an alphoid repeat probe from the chromosome 15 centromere; Choo et al. 1990), D15Z1 (15p specific; Higgins et al. 1985), and two cosmids derived from within proximal 15q, D15S11 and GABRB3 (fig. 2) (Kuwano et al. 1992). A flow-sorted whole-chromosome-15 paint (wcp15) (Telenius et al. 1992) was also used on three of our earliest patients (patients 1, 2, and 10). A total of 10 metaphases were examined after each hybridization, and the number and relative position of the signals were recorded. Signal distribution and intensity from the normal chromosome 15 homologue acted as an internal control for the efficiency and stringency of each hybridization experiment. Analyses were performed by use of a conventional Zeiss epifluorescent Axiophot microscope, and images were captured by a cooled charge-coupled–device camera (Photometrics) and were enhanced and analyzed by use of Digital Scientific (Vysis) Smart Capture software.

Molecular Genetics

DNA was extracted from peripheral blood of patients and their parents by a method based on that of Miller et al. (1988). PCR was performed by use of primer sets for three dinucleotide repeats that map to the PWACR—namely, IR4-3R (D15S11), LS6-1 (D15S113), and γ-aminobutyric acid receptor B3 (GABRB3)—by PCR conditions as described by Mutirangura et al. (1992*a,* 1992*b*). In addition, when FISH analysis gave a normal result, PCR was also performed by use of primer sets for a further nine dinucleotide repeats that map to the PWACR. Products were separated on a 6% polyacrylamide gel and were visualized by autoradiography. Methylation studies were performed on patient 2 and his family, in order to determine the parental origin of his grandmother's duplication. Five micrograms of peripheral-blood DNA was digested with *Hin*dIII and *Hpa*II, was separated on an 0.8% agarose gel, and was analyzed by Southern blot hybridization at 63°C with PW71b (D15S63) (Dittrich et al. 1992). Since *Hpa*II is a methylation-sensitive restriction enzyme, it is unable to cut the maternal chromosome 15, which is methylated in this region; in contrast, the paternal copy is unmethylated. The resulting blot, therefore, has two bands, of 6.0 kb and 4.4 kb, representing the maternal and paternal chromosomes 15, respectively. The probe was labeled with ^{32}P-dCTP by random oligonucleotide priming. The hybridized probe was detected with a Phosphorimage analyzer and was quantitated by use of Fuji PCBAS software (Raytek Scientific).

Comparative Genomic Hybridization (CGH)

CGH was performed on patients 1, 7, and 10 by use of a protocol modified from that of Kallioniemi et al. (1994). Genomic DNA was salt extracted (Miller et al. 1988) and directly labeled by nick translation with Fluorescein-12-dUTP and Texas Red-5-UTP (Dupont), for test and reference DNAs, respectively. Six hundred nanograms of labeled DNA from each of the test and reference sources was used for each of the hybridization mixtures, which were denatured at 72°C for 8 min, applied to normal male target metaphase slides (Vysis), and denatured according to the manufacturers' instructions. After hybridization for 3 d at 37°C, the slides were

Table 1

Clinical and Family Details of Patients with dup(15)(q11q13)

Proband (Age; Sex)	Ascertainment	Phenotype	PWACR?	Inheritance	Parental Phenotype	Grandparental Phenotype
Twins 1a and 1b (2½ years; F)	Fragile X?	Global learning difficulties, especially speech and language	Yes	Maternal, grandpaternal	Normal	?
2 (2 years; M)	Developmental delay	Speech difficulties, hypergonadotrophic hypogonadism	Yes	Maternal, grandmaternal	Mild learning difficulties, fits	Normal
3 (5 years; M)	Severe mental retardation	Severe mental retardation	Yes	Maternal	Mild learning difficulties, speech therapy	…
4 (1½ years; M)	Fragile X?	Developmental delay	Yes	Maternal, grandmaternal	Slow, learning difficulties	Normal
5 (8 years; M)	Fragile X?	Developmental delay, learning difficulties	No	Maternal, grandpaternal	Normal	Normal
6 (3 years; M)	Developmental delay	Communication difficulties	No	Maternal, grandpaternal	Normal	Normal
7 (5 years; M)	Fragile X?	Language disorder, macrocephaly	No	Paternal	Normal	…
8 (3½ years; M)	Fragile X?	Communication problems	No	Paternal	Normal	…
9 (2 years; M)	Short stature	Mild developmental delay, feeding difficulties	No	Maternal, grandmaternal	Normal	Normal
10 (27 years; M)	Congenital heart defect	Ventricular septal defect, pulmonary stenosis, hypoplastic toes	No	Maternal	Normal	…
11 (8 mo; F)	Beckwith Wiedemann syndrome?	No other details	No	Paternal	Normal	…
12 (newborn; M)	+21?	Intrauterine growth retardation, antimongoloid slant, epicanthal folds	No	Paternal	Normal	…
13 (7 mo; F)	Failure to thrive	Sickly child, poor growth	No	Maternal	Normal	…
14 (23 years; F)	Recurrent aborter	Normal	No	?	?	…
15 (28 years; F)	Child with anencephaly	Normal	No	?	?	…
16 (fetus; M)	Serum-increased risk 1:15	Normal	No	Maternal	Normal	…
17 (fetus; M)	Serum-increased risk 1:38	Normal	No	Maternal	Normal	…
18 (fetus; M)	Maternal age	Normal	No	Paternal, grandpaternal	Normal	Normal
19 (fetus; M)	Elevated maternal serum α-fetoprotein	Normal	No	Paternal, grandmaternal	Normal	Normal
20 (fetus; M)	Triple-test risk 1:163	Normal	No	Paternal, grandpaternal	Normal	Normal

washed, counterstained, and inspected under a Zeiss Axioskop fluorescence microscope. Images were captured by a cooled charge-coupled–device camera (Photometrics) and were enhanced and analyzed by use of Quips CGH software (Vysis). Each metaphase used in the analysis was karyotyped, and the green-to-red fluorescence-intensity ratios along the length of each chromosome were calculated. The data from as many as 10 meta-

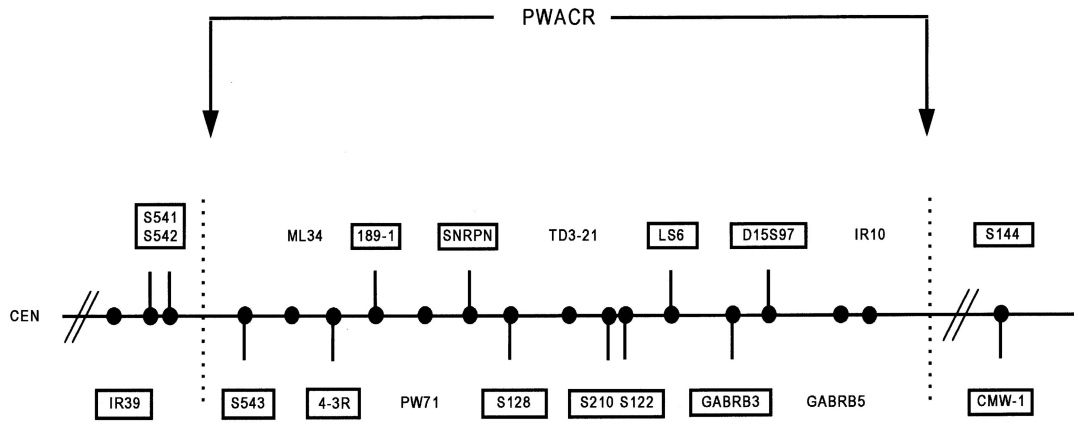

Figure 2 Physical map of 15q11-q13, showing the PWACR and, in rectangles, the relative positions of probes and primers used in FISH and PCR analysis.

phases were combined to give a mean ratio profile for each chromosome.

Results

Cytogenetics

All probands had an apparently identical duplication of the region 15q11-13. C-banding undertaken on three of our earliest patients (patients 1, 2, and 10) indicated that the additional material was not heterochromatic. C-banding was, however, superseded by in situ hybridization with a chromosome 15 centromere probe, for all subsequent patients (see below). In all cases the short arm of the dup(15) was positive for DA/DAPI-banding, excluding the possibility that the dup(15)s had arisen as a result of translocation with other acrocentric chromosomes. Parental bloods were not available for three probands, but the dup(15) was inherited in all 17 remaining families, 10 maternally and 7 paternally (table 1). Analysis of grandparental blood samples from eight families showed that the dup(15) had been inherited from a phenotypically normal grandmother (patients 2, 4, 9, and 19) or grandfather (patients 5, 6, 18, and 20). The grandfather of patients 1a and 1b was deceased, and the grandmother did not carry the dup(15). In addition, patient 2 had two affected sibs and an affected maternal aunt who also carried the dup(15), whereas three unaffected sibs did not; and patient 19 had three phenotypically normal sibs who carried the dup(15), one of whom has transmitted the duplication (detected at amniocentesis) to her current pregnancy.

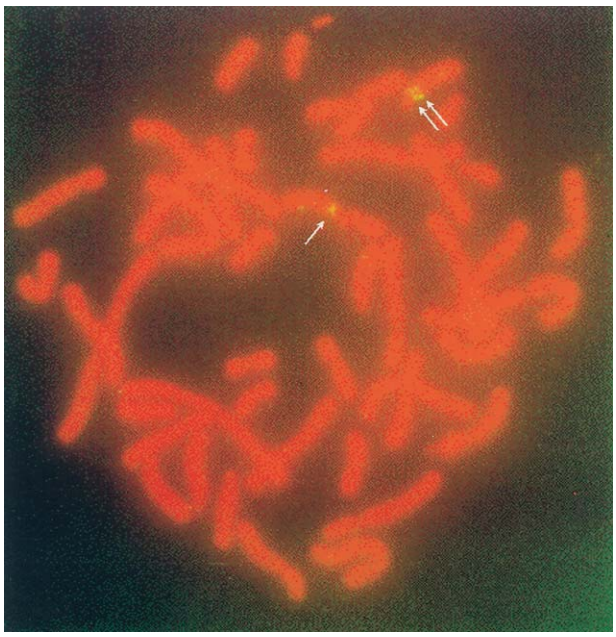

Figure 3 Results of in situ hybridization using D15S11. A single signal can be seen on the normal chromosome 15 (*single proximal arrow*), whereas two signals are seen on the dup(15) (*double arrow*). The single distal signals on both chromosomes indicate an internal control probe at 15q22.

Molecular Cytogenetics

FISH using cosmid probes D15S11 and GABRB3, which map within the PWACR, gave a significantly increased signal, with both probes, in patients 1a, 1b, and 2–4 (fig. 3 and table 1). The pedigrees of these four families are shown in figure 4. In the remaining 16 cases, only signals of equal size were seen on both homologues. In 3 of these 16 cases, FISH using wcp15 suggested that the extra material may be of chromosome 15 origin. However, since it is possible that the additional material was beyond the resolution of the forward-painting tech-

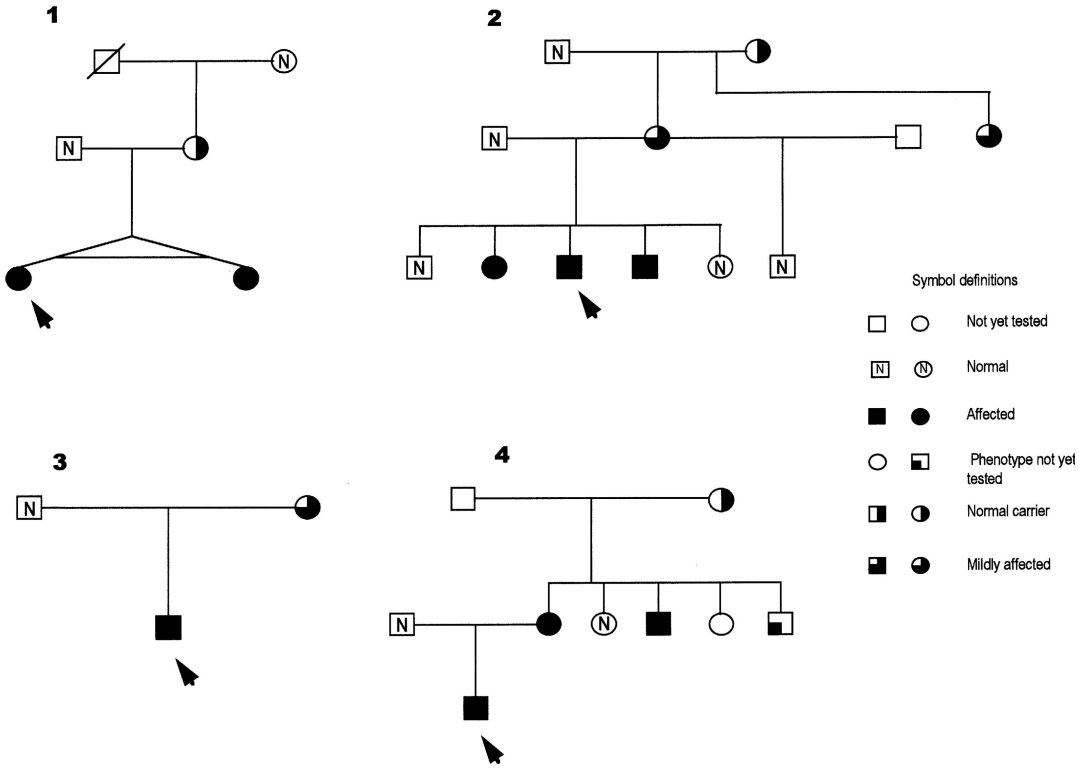

Figure 4 Pedigrees of four patients with a dup(15) including PWACR. Arrows indicate the probands.

nique, this was not repeated on the remaining cases. In addition a probe specific for 15p (D15Z1) gave clear signals on both the normal chromosomes 15 and the dup(15)s, and a chromosome 15 centromere probe (pTRA-20) confirmed that the additional material had not originated from the chromosome 15 centromeric region.

Molecular Genetics

Molecular analysis using primers for three loci from within the PWACR, D15S11, D15S113, and GABRB3 demonstrated the presence of three alleles at each locus in patients 1a, 1b, and 2–4, confirming duplication of this region (fig. 5 and table 2). There was, however, no evidence that the PWACR was included in the duplication in the remaining 16 patients. Further analysis, using 10 additional probes from within proximal 15q, showed that the duplication extended from D15S541 to GABRB5 in patients 1a, 1b, and 2, from D15S541 to GABRB3 in patient 3, and from D15S543 to GABRB5 in patient 4, but again there was no evidence for a PWACR duplication in the remaining 16 patients. Molecular analysis demonstrated that the dup(15) in patient 1 could not have arisen from either of the grandmother's chromosomes 15 and that it was, therefore, paternal in origin. Methylation analysis confirmed the maternal duplication in patient 2, in one of his affected sibs, and in his mother. In contrast, however, a duplication of paternal origin was seen in the grandmother (fig. 6*a*). These results were confirmed by use of a phosphorimage analyzer, to quantitate the dosage of each band (fig. 6*b;* note that the vertical axis on these images varies such that what is important is the *difference* between the peak size of the maternal versus the paternal copies of PW71b, rather than the actual sizes per se).

CGH

CGH undertaken on two patients (patients 7 and 10) with a non-PWACR dup(15) showed an excess of green signal in the 15q11-13 region, represented, on the resulting profile, by a peak that was not seen in a normal control. These results indicated a gain of material of chromosome 15 origin. CGH undertaken on the PWACR-containing duplication of patient 1a gave a similar result. However, the gain of material seen in the latter case appeared more distal than that seen in the former two cases, suggesting that the duplications may have involved different regions within proximal 15q. CGH was repeated in all three patients, with the same results. Examples of the profiles obtained are shown in figure 7.

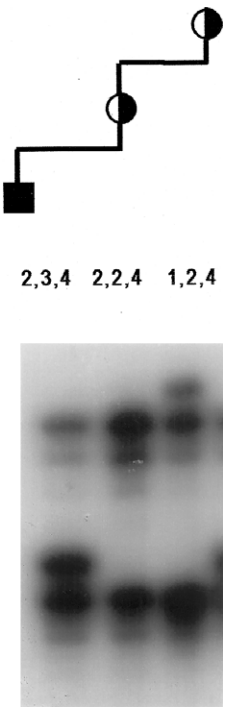

Figure 5 Results of PCR analysis. The primer D15S541 shows the duplication in patient 2, in his mother, and in his grandmother. This result was seen by use of several probes from proximal 15q (see table 2).

Discussion

We have reported the molecular cytogenetic and molecular genetic results from 20 patients with apparently identical interstitial duplications of proximal 15q. Our results demonstrate that molecular investigation using probes from within the PWACR discriminates between two groups of duplications. First, the 16 duplications that do not contain the PWACR are not associated with any specific phenotype and are equally likely to be maternally or paternally inherited. In six of these families the dup(15) was also carried by a phenotypically normal grandparent, and in two of these families it was carried by at least one phenotypically normal sib. These non-PWACR dup(15)s, therefore, appear to be euchromatic variants that may be transmitted through families, without reproductive or clinical effect. Despite the use of 14 probes from within proximal 15q, we were unable to confirm the duplication molecularly. CGH, however, suggested that the additional material in two patients was of proximal 15q origin. Comparison of these CGH results with those from a dup(15) patient including the PWACR indicated that the duplication in the former cases may have been more proximal to the PWACR than they were in the latter case. We await the availability of additional probes from within proximal 15q, to determine the exact nature of these non-PWACR dup(15)s.

Second, the four duplications that did contain the PWACR were associated with developmental delay and speech difficulties, in all four probands. Furthermore, two affected sibs of patient 2 and an affected maternal aunt also carried the dup(15), whereas three unaffected sibs did not. In all four families the dup(15) was maternal in origin. The mothers of patients 2–4 had learning difficulties, with patient 2's mother also having had periods of fits and with patient 3's mother having had speech therapy as a child. The mother of patient 4 also had two brothers with severe learning difficulties. The mother of patients 1a and 1b was, however, unaffected, except for some minor difficulties with mathematics at school. Analysis of her mother's chromosomes (her father was deceased) demonstrated that she did not carry the dup(15), and molecular analysis indicated that the dup(15) could not have arisen from either of her chromosomes 15. Therefore, whether inherited or de novo, the dup(15) in patients 1a and 1b was grandpaternal in origin. Analysis of the grandparents of patients 2 and 4 demonstrated that both duplications were grandmaternally inherited, and methylation analysis showed that the duplication in patient 2's grandmother was paternal in origin. Therefore, in addition to the four probands, at least two of the affected mothers have inherited a maternal dup(15), whereas the duplication in the unaffected mother of patients 1a and 1b and in the unaffected grandmother of patient 2 is paternal in origin, suggesting a parental-origin effect.

A review of the literature revealed very few cases in which an interstitial dup(15) had been investigated molecularly. Bundey et al. (1994) demonstrated the presence of the PWACR in a de novo dup(15) of maternal origin in a 16-year-old male who had severe mental retardation, autism, developmental delay, ataxia, and language and communication impairment, whereas Jalal et al. (1994) demonstrated the absence of the PWACR in an inherited dup(15) in four phenotypically normal individuals. Furthermore, two patients with an interstitial triplication of the PWACR—and, therefore, partial tetrasomy for this region—had a more severe phenotype, suggesting that there is a dosage effect for a gene(s) within this region (Holowinsky et al. 1993; Schinzel et al. 1994; Long et al., in press).

These observations are also comparable with the large number of reported patients with a supernumerary marker of chromosome 15 origin (SMC[15]) who also have additional copies of proximal 15q. Patients with an SMC(15) that does not include the PWACR are usually phenotypically normal, whereas those SMC(15)s including the PWACR are associated with moderate to severe mental retardation and seizures (Robinson et al. 1993*a,* 1993*b;* Cheng et al. 1994; Leanna-Cox et al.

Table 2

Molecular Analysis of dup(15)s, Including PWACR Proximal 15q Probes Used

Patient	D15S541	D15S542	D15S543	D15S11	D15S128	D15S210	D15S122	D15S10	LS6	D15S597	GABRB3	GABRB5	D15S165	D15S144	D15S118	D15S95	CRES
1a	1,1,2	2,3,4	1,1,2	…	…	2,3,4	…	…	2,3,5	…	…	1,3,3	2,3	…	…	…	…
1b	1,1,2	2,3,4	1,1,2	…	…	2,3,4	…	…	2,3,5	…	…	1,3,3	2,3	…	…	…	…
Mother	1,1,2	2,3,5	1,1,2	2,2	1,3	1,2,4	1,2,3	…	2,4,5	1,2,3	1,2,3	2,3,3	3,3	1,2,2	1,2	1,2	1,1
Grandmother	2,2	5,5	2,4	1,2	2,4	1,2	3,3		2,4	-	2,3	2,3	3,3	1,2	1,2	2,3	1,1
2	2,3,4	1,3,4	1,1,2	1,2,2	1,2,3	1,2,3	2,3,4	…	1,1	2,2,3	1,1,3	1,2	1,2	1,2	…	…	…
Mother	2,2,	1,2,3	1,2,2	1,2,2	…	2,3,3	1,2,4	…	1,1	1,2,3	1,1,3	2,3	…	…	…	…	…
Grandmother	1,2,4	1,3	1,2	…	…	2,3,4	…	…	…	…	…	…	…	…	…	…	…
3	1,1,2	2,3,4	1,1	2,3,4	1,2,2	1,2,2	1,2	…	1,2,3	…	1,2,4	…	1,3	1,1	1,1	1,2	1,2
Mother	1,1,2	1,3,4	1,1	1,2,3	1,2,2	1,2,3	1,1,2	…	…	1,2,3	1,2,3	…	1,2	1,1,2	1,2	1,2	…
4	1,2	…	…	1,2,2	…	…	1,2,2	…	…	…	…	1,2,3	…	…	…	…	…
Mother	2,2	…	1,1,2	1,2,2	…	…	1,2,2	…	…	…	…	2,3,4	1,2	…	…	…	…

NOTE.—An individual number is arbitrarily allocated to each allele. Where increased dosage of one of two alleles was found, the number for that allele is entered twice. At occasional loci, clear evidence for additional dosage of either one of two alleles or a single allele was not obtained.

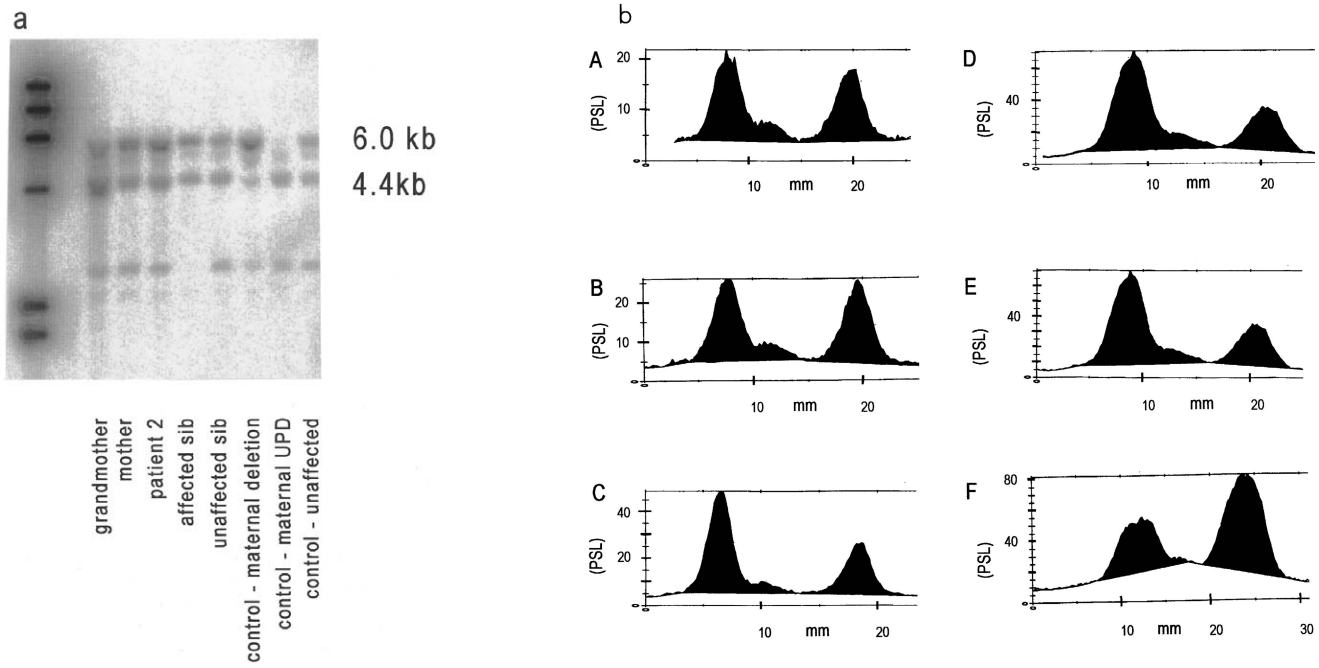

Figure 6 *a,* Methylation status at the D15S63 locus, in family 2 and in controls. The maternal and paternal chromosomes 15 are represented by a 6.0-kb band and a 4.4-kb band, respectively. *b,* Dosage analysis of the methylation status of family 2, showing the parental origin of the duplication. In each panel, the first peak is that of the 6.0-kb methylated maternal chromosome, and the second peak is that of the 4.4-kb unmethylated paternal chromosome. Results are shown for (*A*) an unaffected control (equal peak heights), (*B*) an unaffected sib (equal peak heights), (*C*) an affected sib (increased maternal peak), (*D*) patient 2 (the proband) (increased maternal peak), (*E*) the mother (increased maternal peak), and (*F*) the grandmother (increased paternal peak).

1994; Webb 1994; Crolla et al. 1995; Mignon et al. 1996). In addition, all the de novo SMC(15)s containing the PWACR that were investigated for parental origin were maternal in origin. The more severe phenotype associated with the SMC(15)s, versus that associated with the dup(15)s, may be due to the fact that the former usually contain two copies of the PWACR (Robinson et al. 1993*a*) and that, consequently, such patients are tetrasomic for this region.

The fact that the vast majority of dup(15)s and SMC(15)s including the PWACR are maternal in origin suggests that duplication of the paternal PWACR either (i) is a rare occurrence, (ii) is lethal and rarely survives to term, or (iii) has no phenotypic effect and consequently goes undetected. We have demonstrated a dup(15) including a PWACR of paternal origin in two phenotypically normal individuals, which suggests that an additional copy(copies) of the paternal PWACR may have no adverse effect. This suggestion is supported by the recent report of a complex 5;15 rearrangement found both in a phenotypically normal 33-year-old female referred for recurrent miscarriages and in her phenotypically normal father (McMullen et al. 1996). The rearrangement was found to include a duplication of the PWACR within the derived chromosome 5. However, we cannot rule out the possibility that the absence of a phenotype is due to a position effect—that is, that gene(s) normally transcriptionally active within proximal 15q became inactive in their new position (Bedell et al. 1996). So far, at least four loci expressed only from the paternal chromosome 15 have been identified—the small nuclear ribonucleoprotein N (SNRPN) and three anonymous transcripts: PAR-1(D15S227E) (Ozcelik et al. 1992), PAR-5(D15S226E) (Sutcliffe et al. 1994), and IPW (Wevrick et al. 1994). There are, as yet, no genes known to be transcribed only from the maternal homologue. Consequently, a duplication on a paternally derived chromosome is unlikely to have the same effect as one of maternal origin; however, it is perhaps surprising that there appears to be so little phenotypic effect in the paternal duplications. Since those duplications not including the PWACR are equally of maternal and paternal inheritance, it seems unlikely that the gene(s) involved in these cases originates within the imprinted region. This is consistent with the CGH results, which suggest that the duplications not including the PWACR may be more proximal than the PWACR dup(15)s.

Many of the earliest reports of dup(15) were associated with PWS; but none of our 20 patients appear to have any features specific to this syndrome. However, given the quality of metaphase chromosomes obtained in the early 1980s, in addition to the fact that the ma-

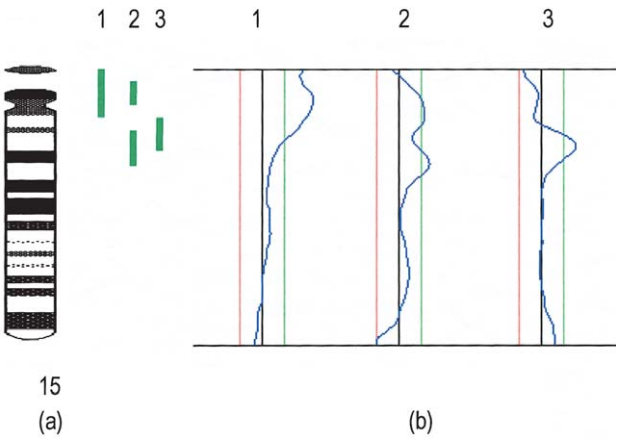

Figure 7 Results of CGH. *a,* Gains of material on chromosome 15, shown as vertical bars along the ideogram. *b,* Average ratio profiles for chromosome 15. For each profile, the vertical midline represents a green:red color ratio of 1:1, and the lines to the left and right represent ratios of 0.9:1 and 1.1:1, respectively. A copy-number change is suggested when a profile lies outside these limits, and a deviation to the right is shown as a gain on the corresponding region of the ideogram. Profile 1 shows results for the normal control. A large deviation is seen in the heterochromatic region. However, variations in apparent copy number in this region cannot be interpreted, because of the absence of a unique sequence–hybridization signal. Profile 2 shows results for patient 1a, who has a dup(15) including the PWACR. In this case two areas of gain can be seen. The upper peak corresponds to the heterochromatic region and is therefore of no significance, whereas the lower peak indicates a gain of proximal 15q material. Profile 3 shows results for patient 7, who has a dup(15) not including the PWACR; the peak shown indicates that the duplication within 15q is more proximal than that seen in patient 1a.

jority of cases did not undergo molecular investigation, it is possible—and perhaps likely—that those patients actually had a deletion of chromosome 15, rather than duplication of the homologous chromosome. Alternatively, PWS in those patients may have been caused by either a submicroscopic deletion of or UPD for chromosome 15, in addition to a dup(15).

During the past 3 years we have detected a dup(15) in ~1/600 blood and ~1/2,000 prenatal samples referred to us for routine cytogenetics. More important, among developmentally delayed individuals we detected a dup(15), including the PWACR, at a frequency of 1/600. Discovery of the imprinted gene(s) associated with this phenotype might well allow direct molecular analysis. It is probable that submicroscopic duplications of this region also exist, which may well account for a proportion of patients referred to the cytogenetics laboratory because of developmental delay and/or learning difficulties for which a fragile X screen proves negative and for which cytogenetic analysis reveals a normal karyotype.

The present study demonstrates that use of molecular probes from within the PWACR, together with parental-origin studies, distinguishes between three types of dup(15)s. First, dup(15)s not including the PWACR are not associated with any specific phenotype and appear to be euchromatic variants, explaining the many reports of phenotypically normal dup(15) carriers in the literature. Second, dup(15)s including the PWACR and maternally inherited are consistently associated with developmental delay and learning and/or speech difficulties in the absence of dysmorphic features. The phenotype is considerably milder than that of patients with an SMC(15) or an interstitial triplicated 15, suggesting a dosage effect for the gene(s) involved. Finally, dup(15)s including the PWACR and paternal in origin have little, if any, phenotypic effect.

Since submission of this paper, Cook et al. (1997) have published concordant results by demonstrating a PWACR duplication in two children with atypical autism and in their phenotypically normal mother. Her duplication was paternal in origin. Although our four PWACR-duplication patients were not described as autistic, the observations of Cook et al. suggest that clinical psychological assessment of all patients may help to extend and refine the phenotypic features associated with the PWACR dup(15)s of maternal origin.

Note added in proof: Since submission of this paper, we have demonstrated that the PWACR dup(15) in the phenotypically normal grandmother of patient 4 is also paternal in origin.

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