# Letters to the Editor

### Inter- and Intrachromosomal Rearrangements Are Both Involved in the Origin of 15q11-q13 Deletions in Prader-Willi Syndrome

*To the Editor:*

Prader-Willi syndrome (PWS) is due to an interstitial de novo deletion at 15q11-q13 in ~70% of cases. The deletion spans a region of ~4 Mb and invariably involves the paternally derived homologue (Robinson et al. 1991). For most patients the distal breakpoint appears to be located within a single YAC (Kuwano et al. 1992), whereas two consistent breakpoint hot spots have been identified on the proximal side; one (class I) lies in the region between the centromere and D15S541/D15S542, and the other (class II) lies between D15S541/D15S542 and D15S543, with each accounting for approximately half of the deletions (Christian et al. 1995).

The relatively high frequency of deletions, significant clustering of the breakpoints in PWS deletion patients, and the finding of a similar location for the breakpoints in small inv dup(15) has led to the hypothesis that small regions in proximal 15q may contain sequences leading to instability (Knoll et al. 1993; Huang et al. 1997). Recently, preliminary data for a low-copy repeat associated with a novel evolutionarily conserved gene family spanning the proximal and distal breakpoint regions has been reported (Ji et al. 1996).

In order to analyze the mechanism underlying deletions in PWS, we genotyped 10 three-generation families of PWS-deletion patients, using microsatellite markers flanking the common deletion region. Each patient was known to be deleted for the interval from D15S11 to GABRB3, by FISH and/or other molecular techniques. Peripheral blood samples were obtained, with appropriate informed consent, from the patient, both parents, and paternal grandparents. DNA was isolated by use of a QIAamp blood kit (Qiagen). We employed markers D15S541 and D15S542, both mapping to YAC A124A3, proximal to the deletion region, and markers D15S165 and D15S1048, both mapping distal to the common deletion region (Hudson et al. 1995). In addition, marker ATC3C11, mapping <1 Mb from the distal deletion breakpoint (S. L. Christian and D. H. Ledbetter, unpublished data), was used. PCR assays were performed as described elsewhere (Christian et al. 1995; Hudson et al. 1995). The results of the microsatellite analysis are shown in table 1, and examples of the analysis performed on two independent families are shown in figure 1.

Three patients were deleted for the D15S541/D15S542 markers, thus being classified as class I–deletion patients. This finding was consistent with the known frequency of the class I breakpoints among PWS patients. The lack of a proximal marker on the deleted allele in these patients precluded assessment of the haplotype for the region comprising the deletion. Of the seven class II patients, five demonstrated a paternal recombination event between the markers flanking the common deletion region. The genetic distance between marker D15S541 and marker D15S165 has been estimated as 17.2 cM in males (Robinson and Lalande 1995). Marker D15S1048 maps 2 cM proximal to D15S165 (Hudson et al. 1995). Therefore, when the genetic distance between D15S541 and D15S165 in male meiosis is taken into account, the identification, in five of seven cases, of a different grandparental origin for the alleles flanking the deletion is significantly different from the expected frequency ($\chi^2 = 12.438$, $P = .0004$). This finding is highly suggestive of an unequal crossover occurring in the paternal meio-

## Table 1

**Microsatellite Analysis of Chromosome 15 Markers in 7 PWS Families**

| | Proximal Markers[a] | | | Distal Markers[a] | | | |
|---|---|---|---|---|---|---|---|
| Family | D15S541 | D15S542 | Grandparental-Allele Inheritance | D15S1048 | D15S165 | Grandparental-Allele Inheritance | Mechanism of Rearrangement |
| 1 | n.t. | GF (ab), GM (cd), F (ad), P(bd), M (bc) | GM | GF (be), GM (ad), F (bd), P (bc), M (bc) | n.t. | GF | Interchromosomal |
| 2 | n.t. | GM (cd), F (bd), P (ab), M (aa) | GF | GM (ab), F (bd), P (bc), M (bc) | GM (bb), F (ab), P (bb), M (ab) | GM | Interchromosomal |
| 3 | n.t. | GF (ab), GM (ac), F (bc), P (cd), M (bd) | GM | GF (ac), GM (bc), F (bc), P (bb), M (bb) | GF (ac), GM (bc), F (bc), P (bb), M (bb) | GM | Intrachromosomal |
| 4 | n.t. | GF (cd), GM (ab), F (ad), P (bd), M (bb) | GF | GF (bd), GM (ad), F (ad), P (ac), M (bc) | GF (de), GM (ac), F (ad), P (ab), M (bc) | GM | Interchromosomal |
| 5 | n.t. | GM (ab), F (bc), P (cd), M (de) | GF | GM (aa), F (ac), P (cc), M (bc) | GM (bb), F (bc), P (ac), M (ac) | GF | Intrachromosomal |
| 6 | GF (ad), GM (ce), F (cd), P (bc), M (bb) | GF (ab), GM (bb), F (bb), P (bb), M (bc) | GM | GF (bd), GM (ce), F (de), P (ad), M (ab) | GF (cd), GM (be), F (bc), P (ac), M (ae) | GF | Interchromosomal |
| 7 | n.t. | GM (cd), F (bd), P (ad), M (ab) | GM | GM (bb), F (bc), P (cc), M (ac) | GM (cd), F (cd), P (bc), M (ab) | GF | Interchromosomal |

[a] n.t. = Not tested; GF = grandfather; GM = grandmother; F = father; P = patient; and M = mother.

sis, at the breakpoint, as being the mechanism leading to the deletion.

Asymmetrical exchanges between nonsister chromatids in meiosis I have previously been demonstrated in humans and are the basis of a number of genetic diseases. When the related sequences are part of tandemly arrayed homologous genes, nonhomologous recombination may lead to the formation of chimeric genes, such as the globin-chain variants in some hemoglobinopathies (Weatherall et al. 1995) and the red-green pigment genes involved in color-vision abnormalities (Nathans et al. 1986). In other instances, the deletion/duplication event may arise from the unequal recombination between repetitive elements interspersed throughout a genomic region. A misalignment between *Alu*-repetitive sequences has been demonstrated in duplications of the LDL-receptor gene (Lehrman et al. 1987) and the hypoxanthine phosphoribosyltransferase gene (Marcus et al. 1993).

Recent studies have demonstrated the presence of two copies of a large repetitive element (CMT1A-REP) flanking the region duplicated in Charcot-Marie-Tooth disease type IA (CMT1A) patients and deleted in patients with hereditary neuropathy with liability to pressure palsies (HNPP), at 17p11 (Pentao et al 1992; Chance et al. 1994). A model of unequal crossing-over between misaligned CMT1A-REP homologues has been proposed for the generation of both the CMT1A duplication and the HNPP deletion. Interestingly, the presence of a *mariner* transposon–like element at the

junction fragment suggests the occurrence of strand-exchange events mediated through the transposase activity (Reiter et al. 1996). Duplications of 15q11-q13 have been reported in only a few instances (reviewed in Clayton-Smith et al. 1993), and it is unclear whether any of these represent the reciprocal event of deletion by unequal crossing-over. The paucity of duplication cases compared with deletions of this region is interesting and indicates either that duplications occur much less frequently or that a milder phenotype causes them to be ascertained much less often.

Conversely, our study also has shown that in two PWS families the data were consistent with an intrachromosomal mechanism being responsible for the deletion. It cannot be ruled out that this observation might be due to a classical crossover occurring, at meiosis I, between D15S541/D15S542 and D15S1048/D15S165, followed or preceded by an unequal homologous recombination. However, a recombination event occurring twice in such a small interval would be unlikely. Intrachromosomal rearrangements have been infrequently demonstrated as a mechanism leading to human diseases. One example is the intrachromosomal recombination occurring at Xq28, between gene A, a small intronless gene within intron 22 of the factor VIII gene, and one of the two copies of gene A located on the same chromosome, 500 kb telomeric to the factor VIII gene, a recombination causing severe hemophilia (Lakich et al. 1993). Molecular studies have demonstrated that this rearrangement arises almost exclusively in male meioses (Rossiter et al.
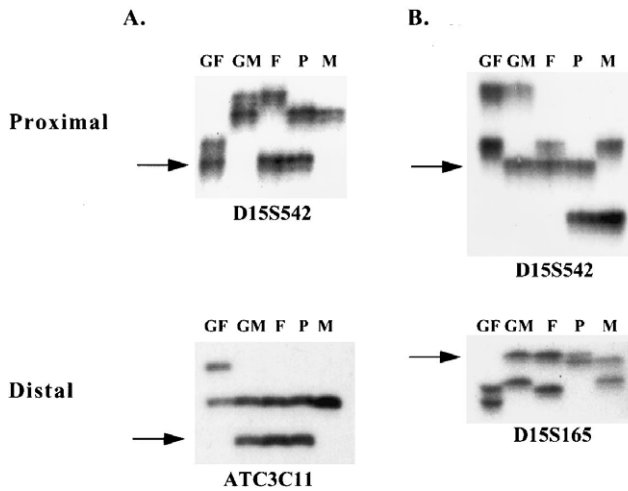
**Figure 1**    Representative microsatellite analysis of two families, illustrating an interchromosomal deletion event and an intrachromosomal deletion event. Data for one proximal and one distal marker are presented. In each example an arrow indicates the inheritance of the patient's paternal chromosome that bears the PWS deletion. *A,* Interchromosomal mechanism is inferred where the proximal marker, D15S542, shows grandpaternal inheritance whereas the distal marker, ATC3C11, shows grandmaternal inheritance of the deleted chromosome. *B,* Intrachromosomal mechanism, demonstrated by the fact that both the proximal marker, D15S542, and the distal marker, D15S165, show grandmaternal inheritance of the deleted chromosome. GF = grandfather; GM = grandmother; F = father; P = patient; and M = mother.

Romeo Carrozzo,[1,2,4] Elena Rossi,[2]
Susan L. Christian,[10] Kirk Kittikamron,[10]
Chiara Livieri,[5] Andrea Corrias,[7]
Lucia Pucci,[8] Alberto Fois,[8] Paolo Simi,[9]
Laura Bosio,[3] Luciano Beccaria,[3]
Orsetta Zuffardi,[2,6] and David H. Ledbetter[10]
[1]*Servizio di Genetica Medica,* [2]*Laboratorio di Citogenetica,* [3]*Clinica Pediatrica III, Universita degli Studi Milano, Ospedale San Raffaele, and* [4]*Telethon Institute of Genetics and Medicine (TIGEM), San Raffaele Biomedical Science Park, Milan;* [5]*Clinica Pediatrica,* [6]*Biologia Generale e Genetica Medica, Universita degli Studi, Pavia;* [7]*Divisione di Endocrinologia Pediatrica, Ospedale Infantile Regina Margherita, Turin;* [8]*Clinica Pediatrica Universita degli Studi, Siena;* [9]*U.O. Azienda Ospedaliera Pisana, Pisa; and* [10]*Center for Medical Genetics, The University of Chicago, Chicago*

1994). The in-*cis* mechanism leading to the deletions in PWS patients can be related either to an exchange of chromosomal material between sister chromatids or to the formation of an intrachromosomal loop, either during meiosis or as a somatic event, followed by an excision of the chromosomal material lying between the recombining regions.

The overall findings of this study are similar to those of Dutly and Schinzel (1996) for Williams syndrome; this latter syndrome is due to a 500-kb interstitial deletion at 7q11.23. Segregation analysis of grandparental markers flanking the deleted region in 15 patients and their parents demonstrated a recombination between grandmaternal and grandpaternal markers on chromosome 7, at the site of the deletion in two of the three cases, whereas an intrachromosomal recombination appeared to have occurred in the remaining cases.

The deletion occurring at the 15q11-q13 band and leading to PWS syndrome appears, therefore, to be due to both inter- and intrachromosomal rearrangements. Given the similar frequency and extent of the maternal deletion at 15q11-q13, which is responsible for 70% of Angelman syndrome cases, it will be interesting to determine whether these two deletion mechanisms are similarly responsible for that disorder.

## References

Chance PF, Abbas N, Lensch MW, Pentao L, Roa BB, Patel PI, Lupski JR (1994) Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. Hum Mol Genet 3:223–228

Christian SL, Robinson WP, Huang B, Mutirangura A, Line MR, Nakao M, Surti U, et al (1995) Molecular characterization of two proximal deletion breakpoint regions in both Prader-Willi and Angelman syndrome patients. Am J Hum Genet 57:40–48

Clayton-Smith J, Webb T, Cheng XJ, Pembrey ME, Malcolm S (1993) Duplication of chromosome 15 in the region 15q11-13 in a patient with developmental delay and ataxia with similarities to Angelman syndrome. J Med Genet 30:529–531

Dutly F, Schinzel A (1996) Unequal interchromosomal rearrangements may result in elastin gene deletions causing the Williams-Beuren syndrome. Hum Mol Genet 5:1893–1898

Huang B, Crolla JA, Christian SL, Wolf-Ledbetter ME, Macha ME, Papenhausen PN, Ledbetter DH (1997) Refined molecular characterization of the breakpoints in small inv dup(15) chromosomes. Hum Genet 99:11–17

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Ji Y, Buiting K, Walkowicz MJ, Johnson DK, Amos-Langraf JM, Stubbs L, Horsthemke B, et al (1996) An evolutionarily conserved gene associated with the common deletion breakpoint regions in the Prader-Willi/Angelman syndromes. Am J Hum Genet Suppl 59:A32

Knoll JHM, Wagstaff J, Lalande M (1993) Cytogenetic and

molecular studies in the Prader-Willi and Angelman syndrome: an overview. Am J Med Genet 46:2–6

Kuwano A, Mutirangura A, Dittrich B, Buiting K, Horsthemke B, Saitoh S, Niikawa N, et al (1992) Molecular dissection of the Prader-Willi/Angelman syndrome region (15q11-13) by YAC cloning and FISH analysis. Hum Mol Genet 1:417–425

Lakich D, Kazazian HH, Antonarakis S, Gitschier J (1993) Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nat Genet 5:236–241

Lehrman MA, Goldstein JL, Russel DW, Brown MS (1987) Duplication of seven exons in LDL receptor gene caused by Alu-Alu recombinatiion in a subject with familial hypercholesterolemia. Cell 48:827–835

Marcus S, Hellegren D, Lambert B, Fallstrom SP, Wahlstrom J (1993) Duplication in the hypoxanthine phosphoribosyltransferase gene caused by Alu-Alu recombination in a patient with Lesch Nyhan syndrome. Hum Genet 90:477–482

Nathans J, Piantanida TP, Eddy RL, Shows TB, Hogness DS (1986) Molecular genetics of inherited variation in human color vision. Science 232:203–210

Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR (1992) Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. Nat Genet 2:292–300

Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny D, Gibbs RA, Lupski JR (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transposon-like element. Nat Genet 12:288–297

Robinson WP, Bottani A, Yagang X, Balakrishnan J, Binkert F, Mächler M, Prader A, et al (1991) Molecular, cytogenetic, and clinical investigation of Prader-Willi syndrome patients. Am J Hum Genet 49:1219–1234

Robinson WP, Lalande M (1995) Sex-specific meiotic recombination in the Prader-Willi/Angelman syndrome imprinted region. Hum Mol Genet 4:801–806

Rossiter JP, Young M, Kimberland ML, Hutter P, Ketterling RP, Gitschier J, Horst J, et al (1994) Factor VIII gene inversions causing severe hemophilia A originate almost exclusively in male germ cells. Hum Mol Genet 3:1035–1039

Weatherall DJ, Clegg JB, Higgs DR, Wood WG (1995) The hemoglobinopathies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 3417–3484

Address for correspondence and reprints: Dr. David H. Ledbetter, Center for Medical Genetics, The University of Chicago, 924 East 57th Street, Room R110, Chicago, IL 60637-1470. E-mail: dhl@genetics.uchicago.edu

———

## DNA Studies of Limb-Girdle Muscular Dystrophy Type 2A in the Amish Exclude a Modifying Mitochondrial Gene and Show No Evidence for a Modifying Nuclear Gene

*To the Editor:*

Limb-girdle muscular dystrophy type 2A (LGMD2A) is characterized by slowly progressive muscle weakness, usually first evident in the pelvic girdle and then spreading to the upper limbs *while sparing facial muscles*. Onset of symptoms is variable (mean age 9 years), and creatine kinase (CK) levels are elevated from early infancy and remain elevated until the individual is well past this age (Jackson and Strehler 1968). Affected individuals are often wheelchair-bound 20–30 years after the onset of symptoms. There is variability in the age of death, and most individuals die in middle age.

The gene for LGMD2A was first linked to chromosome 15 by Beckmann et al. (1991). Allamand et al. (1995) narrowed the region to 15q15.1-q15.3, using large kindreds from the Isle of La Réunion and the northern Indiana Amish. The muscle-specific calcium-activated neutral protease 3 or calpain 3 (*CANP3*) gene, a possible candidate gene in the 15q15.1-q15.3 region, was examined by Richard et al. (1995). Fifteen different mutations, including missense, splice-site, frameshift, and nonsense mutations, were identified in LGMD2A patients, and many others have subsequently been identified. Since the affected patients in La Réunion belong to a genetic isolate presumed to derive from a single ancestor who immigrated to the island during the late 1670s, it was expected that all affected patients from La Réunion would have the same LGMD2A mutation. Paradoxically, six different mutations were identified. This paradox led the investigators to propose digenic inheritance, in which the founder effect is due to an as-yet-unidentified modulating gene (either nuclear or mitochondrial) that permits mutations in *CANP3* to express LGMD2A. This hypothesis does not require the presence of multiple mutations, since the genetic principles of digenic inheritance should apply to all populations with LGMD caused by calpain-3 mutations.

In the Amish of northern Indiana, Richard et al. (1995) identified a single mutation in *CANP3* (CGG→CAG, R769Q) in a homozygous state in affected patients. The authors speculated that the complete penetrance of this disease in the Amish and in the other LGMD2A pedigrees might also be under the control of a second locus. One expectation of the digenic hypothesis would be that some individuals homozygous for the mutation would be clinically unaffected (i.e., CK is normal and there are no physical findings suggestive of LGMD). Because of the possible implications in genetic testing and counseling, we analyzed 580 DNA samples from Amish individuals in one northern Indiana county for the presence of the R769Q mutation, looking for evidence of phenotypically normal R769Q homozygotes.

We initiated the countywide screen by first identifying carrier couples. Appropriate informed consent was obtained from all individuals. In order to identify R769Q carriers in this population, we specifically approached members of 16 previously studied nuclear LGMD2A families from this county. We obtained blood samples