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Chromosome 15 Uniparental Disomy is Not Frequent in Angelman Syndrome

J. H. M. Knoll,*,† K. A. Glatt,* R. D. Nicholls,§ S. Malcolm,‖ and M. Lalande*,‡

*Division of Genetics, Children's Hospital; †Department of Pediatrics, Harvard Medical School; and ‡Howard Hughes Medical Institute, Boston; §Departments of Neuroscience and Pediatrics, University of Florida, Gainesville; and ‖Mothercare Department of Pediatric Genetics, Institute of Child Health, London

Summary

Genetic imprinting has been implicated in the etiology of two clinically distinct but cytogenetically indistinguishable disorders—Angelman syndrome (AS) and Prader-Willi syndrome (PWS). This hypothesis is derived from two lines of evidence. First, while the molecular extents of de novo cytogenetic deletions of chromosome 15q11q13 in AS and PWS patients are the same, the deletions originate from different parental chromosomes. In AS, the deletion occurs in the maternally inherited chromosome 15, while in PWS the deletion is found in the paternally inherited chromosome 15. The second line of evidence comes from the detection of an abnormal parental contribution of 15q11q13 in PWS patients without a cytogenetic and molecular deletion. These patients have two maternal copies and no paternal copy of 15q11q13 (maternal uniparental disomy) instead of one copy from each parent. By qualitative hybridization with chromosome 15q11q13 specific DNA markers, we have now examined DNA samples from 10 AS patients (at least seven of which are familial cases) with no cytogenetic or molecular deletion of chromosome 15q11q13. Inheritance of one maternal copy and one paternal copy of 15q11q13 was observed in each family, suggesting that paternal uniparental disomy of 15q11q13 is not responsible for expression of the AS phenotype in these patients.

Introduction

Angelman syndrome (AS; MIM 23440) (McKusick 1989) is characterized by severe mental retardation, neonatal hypotonia, ataxic movements, and inappropriate laughter. Cytogenetically, two main subsets of patients have been reported: those with a de novo deletion of 15q11q13 and those with apparently normal chromosomes. AS is cytogenetically identical to Prader-Willi syndrome (PWS; MIM 17627) (McKusick 1989), a clinically distinct disorder characterized by neonatal hypotonia and failure to thrive, mild to moderate mental retardation, hypogonadism, hyperphagia, short stature, and small hands and feet. The molecular extents of the de novo deletions in AS and PWS are indistinguishable (Donlon 1988; Knoll et al. 1990) and the deletions have been subdivided into classes I and II (Knoll et al. 1990). Classes I and II share a deletion of chromosome 15q11q13–specific DNA markers 34 (D15S9), 3-21 (D15S10), IR4-3R (D15S11), IR10-1 (D15S12), and 189-1 (D15S13), with class I also deleted for proximal marker IR39d, a subfragment of IR39 (D15S18) (Donlon et al. 1986; Nicholls et al. 1989*a*, 1989*b*). Neither class is deleted for CMW-1 (D15S24) (Rich et al. 1988) or JKGR4 (D15S89; J. H. M. Knoll, K. A. Glatt, and M. Lalande, unpublished data), markers distal to the deletion overlap. While the deletion classes are the same in the two syndromes, the parental origin of the de novo deletions are different. In AS, the deletion occurs on the maternally derived chromosome 15 (Knoll et al. 1989), and in PWS, the deletion occurs on the paternally derived chromosome 15 (Butler and Palmer 1983). These findings implicate genetic imprinting, a phenomenon in which the expression of genes is influenced by their parental origin, as a potential mechanism for the etiology of the AS and PWS phenotypes.

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Address for correspondence and reprints: Dr. Marc Lalande, Division of Genetics, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115.

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Genetic imprinting has been most extensively studied in the mouse. Interestingly, maternal or paternal uniparental disomy and the reciprocal deficiency for certain chromosomal segments in the mouse can result either in normal development, embryonic lethality, and/or phenotypic effects in size, shape, and behavior (Solter 1988; Cattanach and Beechey, in press). Uniparental disomy has now been identified in several human genetic disorders (Reik 1989; Hall 1990) including PWS, in which we observed maternal uniparental disomy of chromosome 15q11q13 in patients with no detectable deletion (Nicholls et al. 1989*a*). Utilizing 8 chromosome 15q11q13–specific DNA markers, we have now determined whether paternal uniparental disomy occurs in AS patients lacking detectable cytogenetic and molecular deletions.

Material and Methods

Patients were diagnosed with AS at either the Children's Hospital, Boston; the Clinical Genetics and Dysmorphology Program at Dartmouth Medical School; the Institute of Child Health, London; or the Division of Genetics, University of Florida, Gainesville. Three families had a single affected child (WJK113, WJK121, and F5173), and two affected sibs were in each of the other five families. Both affected sibs were available from two of the families (WJK1, WJK4 and WJK14, WJK15), and one affected sib was available from each of the other three families (A4954, B5211, C5132). WJK113 and WJK121 have been previously reported (patients 4 and 5, respectively) (Williams et al. 1989), as have the two complete sib pairs (WJK1, WJK4 [Knoll et al. 1990; Pashayan et al. 1982] and WJK14, WJK15 [Knoll et al. 1990]).

DNA isolated from peripheral blood or lymphoblastoid cell lines (Aldridge et al. 1984) of these 10 AS patients and their parents was digested with *Rsa*I, *Sca*I, *Taq*I, and *Sac*I (New England Biolabs). Digested DNA fragments were separated by agarose gel electrophoresis, transferred to nylon membrane (Hybond-N; Amersham) and hybridized with radiolabeled (Feinberg and Vogelstein 1983) cloned DNA segments D15S9 (34), D15S10 (3-21), D15S11 (IR4-3R), D15S12 (IR10-1), D15S13 (189-1), D15S17 (IR29-1), D15S18 (IR39d, a subfragment of IR39) (Donlon et al. 1986; Nicholls et al. 1989*a*, 1989*b*), D15S24 (CMW-1) (Rich et al. 1988), and D15S89 (JKGR4) as described elsewhere (Knoll et al. 1989). JKGR4 is a 1.2-kb *Eco*RI chromosome 15q11q13–specific DNA segment, isolated from a flow-sorted inverted/duplicated chromosome 15 lambda GT10 phage library (J. H. M. Knoll, K. A. Glatt, and M. Lalande, unpublished data).

Results

Maternal and paternal contributions for DNA markers within (IR4-3R) and outside (JKGR4) the critical region of deletion overlap are shown in figure 1, *top* and *bottom*, respectively. IR4-3R detects a two-allele RFLP on *Rsa*I-digested DNA as shown in figure 1, *top*. AS patient WJK113 (lane 3) is heterozygous for a paternal 1.0-kb allele (WJK111; lane 1) and a maternal 1.2-kb allele (WJK112; lane 2). AS patient WJK121 (lane 5) is also heterozygous for IR4-3R, indicating the presence of a maternal 1.2-kb allele since the father (WJK120; lane 4) is homozygous for the 1.0-kb allele. Maternal contributions for IR4-3R were also demonstrated in the AS sib pair, WJK1 and WJK4 (table 1).

JKGR4 detects a four-allele RFLP on *Taq*I-digested DNA as shown in figure 1, *bottom*. AS patient C5132 (lane 2) is heterozygous for a paternal 3.1-kb allele (C5134; lane 1) and a maternal 3.0-kb allele (C5133; not shown). In the WJK1-4 family, parental contributions of JKGR4 are not informative as to parental origin but reveal that one of the affected children is a recombinant (see below). AS patient WJK1 is homozygous for the 3.0-kb allele (lane 5). His affected brother, WJK4, and his parents are heterozygous for a 3.0-kb allele and a 1.0-kb allele (lanes 6–8). In AS patients A4954 and F5173, maternal and paternal contributions for JKGR4 could also be determined.

A summary of the molecular data from nondeletion AS patients and their families is presented in table 1. Allele sizes are included in the table and single allele sizes represent homozygosity (two copies per diploid genome) at the locus. The cloned markers that detect a maternal and a paternal contribution or at least a maternal contribution are shown in boldface. In all families, maternal and paternal contributions were demonstrated for at least one locus within 15q11q13. Maternal contributions were observed with four of the five markers within the critical region of deletion overlap of classes I and II (3-21, 189-1, 34, IR4-3R) and all of the DNA markers outside the critical region of deletion overlap (IR39d, JKGR4, CMW-1).

Discussion

Paternal uniparental disomy in these 10 cytogenetically normal AS patients has not been detected. Since maternal uniparental disomy for the same region results

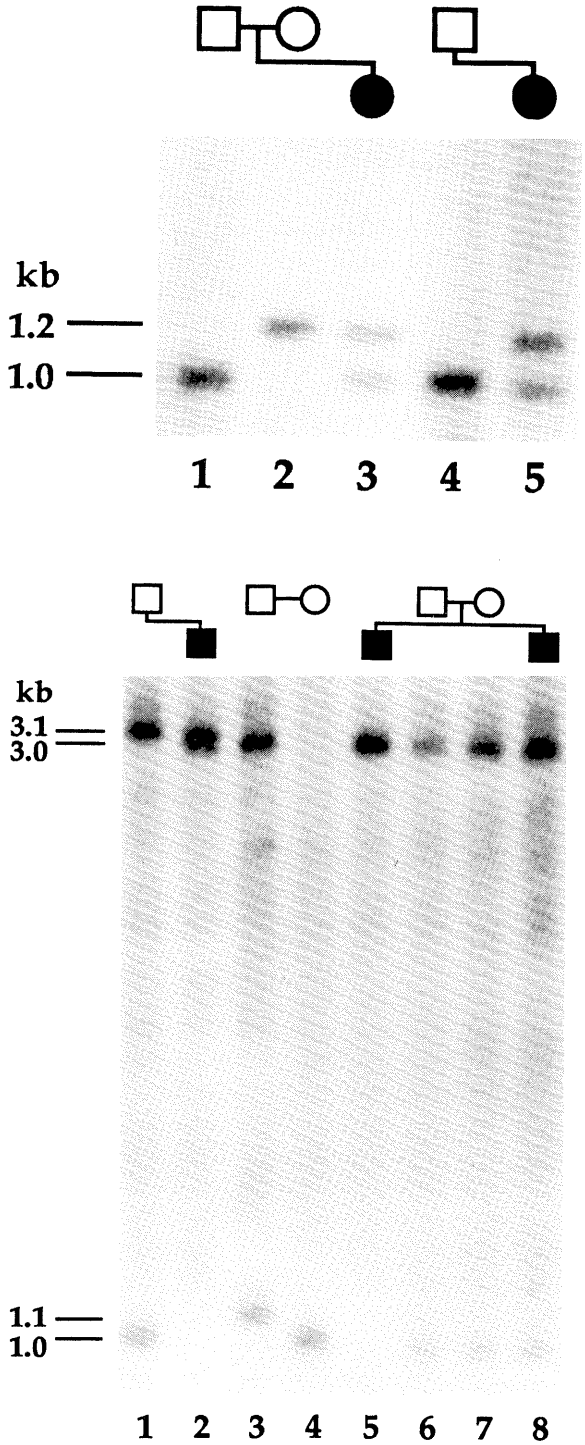

Figure 1 Paternal and maternal contributions of chromosome 15q11q13–specific probes in nondeletion AS families. *Top,* Segregation of *RsaI* alleles by probe IR4-3R. AS patient WJK113 (lane 3) inherits both a paternal 1.2-kb allele (WJK111; lane 1) and a maternal 1.0-kb allele (WJK112; lane 2). AS patient WJK121 (lane 5) is heterozygous for a paternal 1.0-kb allele (WJK120; lane 4) and a maternal 1.2-kb allele. *Bottom,* Segregation of *TaqI* alleles detected by probe JKGR4. AS patient C5132 (lane 2) inherits both a paternal 3.1-kb allele (C5134; lane 1) and a maternal 3.0-kb allele. Affected AS sibs, WJK1 (lane 5) and WJK4 (lane 8), both inherit a 3.0-kb allele. WJK1 is homozygous for a 3.0-kb allele, and WJK4, like his father (WJK2; lane 6) and mother (WJK3; lane 7) is heterozygous for a 3.0-kb and a 1.0-kb allele. WJK104 (lane 3) and WJK105 (lane 4) are parents of a deletion AS patient and serve as controls to demonstrate 3.0-, 1.1-, and 1.0-kb alleles.

in PWS, these findings suggest that paternal uniparental disomy may be incompatible with early development or result in a phenotype different from Angelman syndrome. It is possible that uniparental disomy may occur in sporadic nondeletion cases, and its absence in this study may reflect our patient population, as only three of the 10 patients are potentially sporadic cases. The absence of paternal uniparental disomy in this nondeletion AS population indicates that other mechanisms are responsible for disease expression. One hypothesis is that nondeletion AS is due to a recessive mutation in 15q11q13 and that each parent must be a carrier for a recessive mutant allele (Baraitser et al. 1987; Willems et al. 1987). Since affected siblings WJK1 and WJK4 each received different maternal and different paternal alleles for D15S13 (189-1) and would therefore require recombination between D15S13 and the AS gene on both parental chromosomes 15 (Knoll et al. 1990), this hypothesis seems unlikely.

A second possibility is that AS individuals inherit a mutant maternal allele and a normal paternal allele of a gene in 15q11q13. This model would be consistent with the known maternal origin of de novo AS deletions (Knoll et al. 1989). Affected siblings WJK14 and WJK15 each received the same maternal chromosome 15 region for DNA markers that span 15q11q13-IR39d, 189-1 and CMW-1, and the same paternal chromosome 15 region for IR4-3R and 34 but a different paternal chromosome 15 region for JKGR4. In the other affected sib pair, cytogenetic heteromorphisms revealed that WJK1 and WJK4 each received a different paternal chromosome 15 short arm (Knoll et al. 1990). Molecular analysis showed different maternal and different paternal chromosome 15 contributions for DNA marker 189-1 (see above) but the same contribution from one of the parents for DNA marker JKGR4. These results indicate recombination between the loci for 189–1 and JKGR4 on either the maternal or paternal chromosome 15 in the WJK1/WJK4 sib pair. Whether recombination occurred on the maternal or the paternal chromosome 15 in this family cannot be tested until more in-

Table 1

Molecular Analyses of AS Patients and Their Families

Subject	Phenotype	DNA Sequence							
		IR39d	189-1	IR43R	34	3-21[a]	IR10-1	JKGR4	CMW1[a]
Isolated cases:[b]									
WJK113...	AS	8.5	3.8	**1.2/1**	6.5/6.3	9	16/12.5	1.1/1	A
WJK112...	Mother	8.5	3.8	1.2	6.5	9	16	1	A
WJK111...	Father	14/8.5	3.8	1	6.5/6.3	9/8.9	16/12.5	1.1/1	A
WJK121...	AS	8.5	3.8	**1.2/1**	6.5	**9/8.9**	16	1	A
WJK120...	Father	8.5	3.8	1	6.5/6.3	8.9/8.2	17.5/16	1.1/1	A
F5173.....	AS	...	**3.8/2.0**	1.2/1	6.5	9	16/12.5	**1.1/1**	A/**B**
F5174.....	Mother	...	2.0	1.2	6.5/6.3	9/8.9	16	1.1	A/B
F5175.....	Father	...	3.8	1.2/1	6.5/6.3	9/8.9	16/12.5	1	A
Familial cases:									
WJK1	AS	14/8.5	2	**1.2/1**	6.5	9	16	3	A
WJK4	AS	14/8.5	3.8	**1.2/1**	6.5	9	16	3/1	A
WJK3	Mother	14/8.5	3.8/2	1	6.5	9	16	3/1	A
WJK2	Father	14/8.5	3.8/2	1.2	6.5	9	16	3/1	A
WJK14.....	AS	**14**/8.5	3.8	1	6.5	9	16	3/1	B
WJK15.....	AS	**14**/8.5	3.8	1	6.5	9	16	1	B
WJK16.....	Mother	14/8.5	3.8/2	1	6.5	9	16	1	A/B
WJK17.....	Father	8.5	3.8	1.2/1	6.5/6.3	9	16	3/1	B
A4954	AS	8.5	3.8	1.2/1	6.5	9/8.2	16	**1.1/1**	A
A4955	Mother	8.5	3.8	1	6.5/6.3	9	16	1	A
A4956	Father	8.5	3.8	1.2/1	6.5/6.3	9/8.2	16/17.5	1.1	A/B
B5211.....	AS	8.5	3.8/**2**	1.2	6.5/**6.3**	9	16	...	**A/C**
B5213.....	Mother	8.5	3.8/2	1.2/1	6.5/6.3	9	16	...	A/B
B5214.....	Father	8.5	3.8	1.2	6.5	9	16	...	C
C5132	AS	14/8.5	3.8	**1.2**/1	6.5/6.3	9/8.9	16	**3.1/3**	**B**/C
C5133	Mother	14/8.5	3.8	1.2/1	6.3	9	16/12.5	3/1.1	B/C
C5134	Father	14	3.8	1	6.5/6.3	9/8.9	16	3.1/1	A/C

Note.—DNA sequences read from centromere at left to telomere at right. Entries in boldface represent alleles in which either a maternal contribution or a maternal contribution and paternal contribution is apparent. Alleles for CMW-1 have been arbitrarily assigned A, B, or C within a family since multiple alleles with slight size variation are observed.

[a] The relative order of probes enclosed in boxes is unknown.

[b] One affected individual per family; could be sporadic or familial.

formative markers at other loci within the critical region are isolated. If the recombination event in this family occurred on the maternal chromosome, this would be consistent with an AS mutation received from the mother in both siblings. If a single mutated allele is sufficient for syndrome expression when inherited through the mother, then phenotypically normal women with AS children have high levels of gonadal mutation, have germinal mosaicism, or have inherited the mutant gene from their fathers.

A third hypothesis to explain the results is that a non-chromosome 15q11q13 locus is producing the AS phenotype. While the nondeletion and deletion AS patients appear clinically indistinguishable, genetic heterogeneity as has been described in other disorders such as tuberous sclerosis (Haines et al. 1989; Sampson et al. 1989; Smith et al. 1989), aniridia (Francke et al. 1979; Ferrell et al. 1987), and Wilms tumor (Grundy et al. 1988; Huff et al. 1988) could exist. Further molecular and clinical investigations are needed to determine this.

Since uniparental disomy occurs frequently in nondeletion PWS (Nicholls et al. 1989*a*) and infrequently in nondeletion AS, one might postulate the ratio of deletion to nondeletion cases to be different for the two

syndromes. Consistent with this postulate, a higher frequency of 15q11q13 deletions in AS than in PWS has been observed in high-resolution chromosome analyses (Magenis et al. 1990). In addition, the relative frequency of nondeletion cases caused by mechanisms other than uniparental disomy should be greater in AS than in PWS and may explain the apparently greater number of AS familial cases, all of which are nondeletion. Such considerations are of clinical significance. While a deletion of chromosome 15q11q13 is a de novo event and the risk of recurrence is low, the risk of recurrence in nondeletion cases is unknown. To date, all familial AS cases have cytogenetically normal chromosomes, and it will be important to determine whether apparently sporadic and familial nondeletion cases have the same or different etiologies. The recurrence risks and the mechanism(s) resulting in the expression of the AS phenotype will not be resolved until the gene or genes responsible are identified.

Note added in proof: Uniparental disomy in 15q11q13 has now been excluded in one additional nondeletion AS patient. One of us (S. Malcolm) has recently observed paternal uniparental disomy in two AS patients by using DNA markers CMW-1 and CMS620, which are distal to the critical region. These results suggest that uniparental disomy occurs in nondeletion AS but is infrequent.

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References

Aldridge J, Kunkel L, Bruns G, Tantravahi U, Lalande M, Brewster T, Moreau E, et al (1984) A strategy to reveal high-frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546–564

Baraitser M, Patton M, Lam STS, Brett EM, Wilson J (1987) The Angelman (happy puppet) syndrome: is it autosomal recessive? Clin Genet 31:323–330

Butler MG, Palmer CG (1983) Parental origin of chromosome 15 deletion in Prader-Willi syndrome. Lancet 1:1285–1286

Cattanach BM, Beechey CV. Chromosome imprinting phenomena in mice and indications in man. Chromosomes Today (in press)

Donlon TA (1988) Similar molecular deletions on chromosome 15q11.2 are encountered in both the Prader-Willi and Angelman syndromes. Hum Genet 80:322–328

Donlon TA, Lalande M, Wyman A, Bruns G, Latt SA (1986) Isolation of molecular probes associated with the chromosome 15 instability in the Prader-Willi syndrome. Proc Natl Acad Sci USA 83:4408–4412 (correction in 83:6964)

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Ferrell RE, Chakravarti A, Antonarakis S, Antoszyk JH, Hittner HM (1987) Aniridia 1 update of linkage to ACP. Cytogenet Cell Genet 46:614

Francke U, Holmes LB, Atkins L, Riccardi VM (1979) Aniridia-Wilms' tumor association: evidence for specific deletion of 11p13. Cytogenet Cell Genet 24:185–192

Grundy P, Koufos A, Morgan K, Li FP, Meadows AT, Cavenee WK (1988) Familial predisposition to Wilms' tumor does not map to the short arm of chromosome 11. Nature 336:374–376

Haines JL, Amos J, Atwood J, Bech-Hansen NT, Burley M, Conneally PM, Connor JM, et al (1989) Linkage heterogeneity in tuberous sclerosis. Cytogenet Cell Genet 51:1010

Hall JG (1990) Genomic imprinting: review and relevance to human diseases. Am J Hum Genet 46:857–873

Huff V, Compton DA, Chao LY, Strong LC, Geiser CF, Saunders GF (1988) Lack of linkage of familial Wilms' tumor to chromosomal band 11p13. Nature 336:377–378

Knoll JHM, Nicholls RD, Magenis RE, Glatt K, Graham JM Jr, Kaplan L, Lalande M (1990) Angelman syndrome: three molecular classes identified with chromosome 15q11q13–specific DNA markers. Am J Hum Genet 47: 149–154

Knoll JHM, Nicholls RD, Magenis RE, Graham JM Jr, Lalande M, Latt SA (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32: 285–290

McKusick VA (1989) Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes, 9th ed. Johns Hopkins University Press, Baltimore

Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown M, Budden S, Cohen R, et al (1990) Comparison of the 15q deletion in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin and clinical consequences. Am J Med Genet 35:333–349

Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M (1989*a*) Genomic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342:281–285

Nicholls RD, Knoll JHM, Glatt K, Hersh J, Brewster T, Graham JM Jr, Wurster-Hill D, et al (1989*b*). Restriction fragment length polymorphism within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Am J Med Genet 33:66–77

Pashayan HM, Singer W, Bove C, Eisenber E, Seto B (1982) The Angelman syndrome in two brothers. Am J Med Genet 13:295–298

Reik W (1989) Genomic imprinting and genetic disorders in man. Trends Genet 5:331–336

Rich DC, Witkowski CM, Summers KM, vanTuinen P, Ledbetter DH (1988) Highly polymorphic locus D15S25 (CMW-1) maps to 15pter-q13. Nucleic Acis Res 16:8740

Sampson JR, Yates JRW, Pirrit LA, Fleury P, Winship I, Beighton P, Connor JM (1989) Tuberous sclerosis: evidence for genetic heterogeneity with one locus on 9q. Cytogenet Cell Genet 51:1073

Smith M, Dumaras K, Baumann R, Yoshiyama K, Nakamura Y, Julier C, Trofatter JA, et al (1989) Evidence for genetic heterogeneity in tuberous sclerosis: one gene maps to the 9q34 and a second gene maps in the 11q22-11q23 region. Cytogenet Cell Genet 51:1082

Solter D (1988) Differential imprinting and expression of maternal and paternal genomes. Annu Rev Genet 22: 127–146

Willems PJ, Dijkstra I, Brouwer OF, Smit GPA (1987) Recurrence risk in the Angelman ("happy puppet") syndrome. Am J Med Genet 27:773–780

Williams CA, Gray BA, Hendrickson JE, Stone JW, Cantu ES (1989) Incidence of 15q deletion in the Angelman syndrome: a survey of twelve affected persons. Am J Med Genet 32:339–345