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Cytogenetic and Molecular Studies in the Prader-Willi and Angelman Syndromes: An Overview

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The majority of patients with Angelman syndrome and Prader-Willi syndrome have a cytogenetic and molecular deletion of chromosome 15q11q13 with the primary difference being in the parental origin of deletion. Our current understanding of the cytogenetics and molecular genetics of these 2 clinically distinct syndromes will be discussed in this review. © 1993 Wiley-Liss, Inc.

KEY WORDS: chromosome 15, Angelman syndrome, Prader-Willi syndrome

INTRODUCTION

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are clinically distinct syndromes with a shared cytogenetic deletion of chromosome 15q11q13 in most patients [Ledbetter et al., 1981, 1982; Butler et al., 1986; Kaplan et al., 1987; Magenis et al., 1987; Pembrey et al., 1989; Williams et al., 1989]. Clinical manifestations in PWS include reduced fetal activity, neonatal hypotonia and feeding problems, mental retardation, childhood obesity, short stature, small hands and feet, hypogonadism, and an abnormal facial appearance [Prader et al., 1956]. AS is characterized by ataxic movements, seizures, severe mental retardation, absent speech, frequent laughter, and specific facial characteristics [Angelman, 1965]. Because of the clinical distinctness and the apparent cytogenetic similarity of AS and PWS, these 2 syndromes have recently been the subject of intensive investigations. Our current understanding of the cytogenetics and molecular genetics of these 2 syndromes will be discussed in this review.

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CYTOGENETICS

The most common cytogenetic abnormality associated with AS and PWS is an interstitial deletion of 15q11q13 [Ledbetter et al., 1982; Butler et al., 1986; Pembrey et al., 1989; Williams et al., 1989]. These deletions, found in approximately 60% of AS and PWS patients are de novo and familial recurrences have not been confirmed. About one third of patients have apparently normal chromosome 15s and include PWS and AS patients with affected sibs [Lubinsky et al., 1987; Knoll et al., 1990; Hamabe et al., 1991b] and the remaining 5% have other chromosome abnormalities involving 15q11q13. These abnormalities include inversions, duplications, translocations, and small bisatellited additional chromosomes (SBACs). Chromosome 15, unlike the other acrocentric chromosomes, is seldom involved in Robertsonian translocations, although chromosome 15 Robertsonian translocations are overrepresented in the PWS population [Mattei et al., 1984].

When PWS or AS is suspected, it is necessary to perform chromosome analysis at the 550 band stage and preferably the 850 band level. Band q12 splits at the 850 band stage as shown in Figure 1 and this splitting does not represent a duplication. This observation contrasts with that of ISCN 1985 [Harnden and Klinger, 1985] but confirms that of Magenis et al. [1990]. Microscopically, AS deletions appear larger than PWS deletions [Magenis et al., 1990; J.H.M.K. and M.L., personal observations] but no corresponding differences in the molecular extents of the deletions have been reported [Knoll et al., 1989, 1990]; this may reflect incomplete coverage of 15q11q13 with existing DNA probes and/or differential homologue condensation. A cytogenetic deletion of proximal 15q is difficult to detect reliably because of the heteromorphic nature of 15q11.2, the size of the region, its variability in homologue condensation, and its proximity to the centromere and resulting tendency to bend. Given the difficulties in microscopically assessing 15q11q13, fluorescence in situ hybridization (FISH) with DNA probes specific to the deletion region greatly facilitates the detection of a deletion. This approach has the added advantage of confirming a deletion in inter-

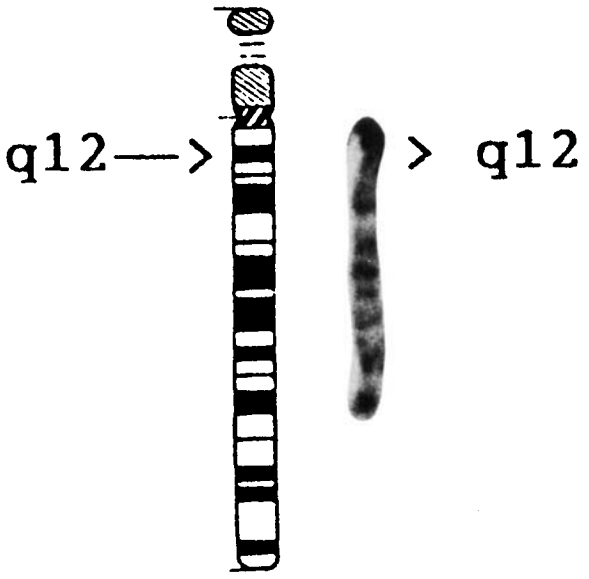

Fig. 1. High resolution GTG-banded normal chromosome 15 and idiogram at the 850 band level. Band q12 splits at this level of resolution.

phase nuclei. An example of this approach using cloned markers specific for 15q11q13 is presented in Figure 2.

Parental origin of the 15s has been assessed in PWS and AS families using cytogenetic polymorphisms of chromosome 15. These polymorphisms, including variations in the nucleolar organizing regions, the size of the short arms, and the fluorescence of the centromeres have documented that the deletion is on the paternally derived chromosome 15 in PWS [Butler and Palmer, 1983; Butler et al., 1986; Mattei et al., 1984; Niikawa and Ishikiriyama, 1985] and confirmed previous molecular findings [Knoll et al., 1989] that the deletion is on the maternally derived 15 in AS [Magenis et al., 1990; Williams et al., 1990].

The variety and frequency of abnormalities involving proximal 15q suggest a vulnerability of this region to rearrangements. Higher rates of cytological meiotic chiasma [Saadallah and Hultén, 1983] and sister chromatid exchanges [Wenger et al., 1989] have been observed. The nature of the chromatin in chromosome 15 differs from the other acrocentrics in its cytochemical properties. Its constitutive heterochromatin, composed of all of the satellite DNAs, is rich in 5-methylcytosine [Okamoto et al., 1981], a finding consistent with 5-methylcytidine antibody specificity [Miller et al., 1974], distamycin/DAPI stainability [Schweizer et al., 1978; Smeets et al., 1991], and failure to condense after treatment with 5-azacytidine [Schmid et al., 1984]. Specific DNA sequences leading to instability of this region have been suggested [Donlon et al., 1986] and require further investigation.

MOLECULAR GENETICS

Chromosome 15q11q13 specific markers have been isolated from genomic libraries constructed from flow sorted SBACs [Donlon et al., 1986; J.H.M.K. and M.L., in preparation], hybrid cell lines [Tasset et al., 1988],

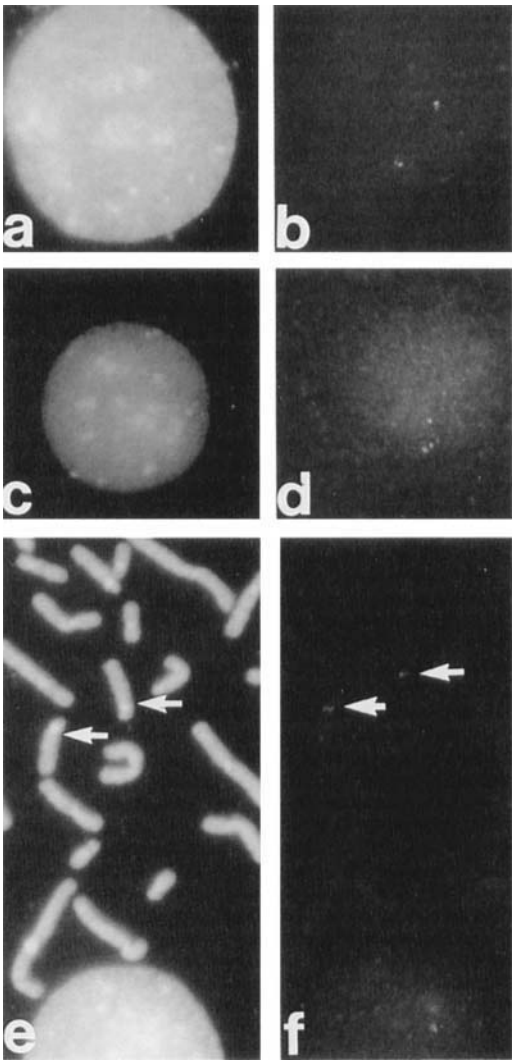

Fig. 2. Two-color FISH with 2 or more chromosome 15q11q13 specific probes on interphase nuclei (**a—d**) and metaphase chromosomes. Large insert phage clone for IR4-3R, 34, 3–21, and GABRB3 were labeled with either digoxigenin-11-dUTP or biotin-16-dUTP, hybridized, and detected as previously described [Lawrence et al., 1990]. Efficiency of hybridization for each probe was determined prior to multiple hybridizations. Two chromosomes 15q11q13 are evident in the interphase nucleus of a control individual (**a,b**), while a single 15q11q13 (three probe hybridization) is present in an AS patient with a cytogenetic and molecular deletion (**c,d**). Specificity of 2 of the clones to chromosome 15q11q13 is shown (**e,f**). DAPI fluorescence images are shown (**a,c,e**) and red and green fluorescence were viewed through a double-band pass filter (Omega Optical). For color reproduction of this figure, please see page 47 of this issue.

and from microdissected 15q11q13 chromosomal regions [Buiting et al., 1990]. Most studies have utilized cloned markers (3-21, IR39d, IR4-3R, IR10-1, 34, 189-1) derived from the first SBAC library [Donlon et al., 1986]. These markers have been used to analyze DNA from AS and PWS patients quantitatively and qualitatively. By quantitative analyses, 3 main molecular classes have emerged [Tantravahi et al., 1989; Knoll et al., 1990]. Two of these classes represent large molecular deletions that differ only by the presence or absence of one centromere proximal marker (IR39d) and the third

class represents a molecular class in which no deletion is detected [Knoll et al., 1990]. The 2 deletion classes correspond to patients with cytogenetically visible deletions and the third class corresponds to patients with normal chromosomes. The presence or absence and extent of deletion within each syndrome appear to have relatively little effect on the phenotype with the possible exception, at least for PWS, of the association of a deletion with hypopigmentation [Wiesner et al., 1987; Butler, 1989].

Recently, a maternally transmitted deletion of marker 3–21 in 3 AS sibs was reported [Hamabe et al., 1991b]. Its presence in several relatives provides the first clear evidence of a submicroscopic deletion. Other submicroscopic rearrangements including duplications, deletions combined with duplications, and discontinuous deletions have been reported [Gregory et al., 1990; Hamabe et al., 1991a]. However, these have been single cases and the interpretations may reflect the limitations and difficulties of quantitative hybridization and high resolution chromosome analyses. To confirm these rearrangements and their submicroscopic nature, cytogenetic deletion detection by FISH would be useful and could serve to reduce the critical regions of AS and PWS.

Restriction fragment length polymorphisms (RFLPs) for the cloned DNA markers have shown that the deletion is on the maternally derived 15 in AS [Knoll et al., 1989; Williams et al., 1990] and have confirmed the paternal origin of the deleted 15 in PWS [Nicholls et al., 1989a; Hamabe et al., 1991a; Robinson et al., 1991]. This difference in parental origin was the first genetic evidence that 15q11q13 may be imprinted. Additional evidence in favor of imprinting came from the interesting observation of Nicholls et al. [1989b] that nondeletion PWS patients have 2 maternal copies and no paternal copy of 15q11q13 (maternal uniparental disomy). Interestingly, RFLP analyses in all confirmed nondeletion PWS patients have shown maternal uniparental inheritance of chromosome 15 [Robinson et al., 1991; Hamabe et al., 1991a; J. H. M. K. and M. L., unpublished data] while similar analyses in nondeletion AS patients have demonstrated both a maternal and a paternal copy of 15q11q13 in most patients [Knoll et al., 1991; Hamabe et al., 1991b]. Only a small fraction of nondeletion AS patients has paternal uniparental disomy [Malcolm et al., 1991]. This observation provides yet another genetic difference between the 2 syndromes. Imprinting of this region is further substantiated by the maternal transmission of a submicroscopic deletion to 3 AS children, where no phenotype is associated with paternal transmission of the same deletion [Hamabe et al., 1991b]; and by the occurrence within an extended family of both AS and PWS dependent on whether an unbalanced translocation involving chromosome 15 was maternally or paternally inherited [Hultén et al., 1991].

ETIOLOGY

Gene(s) in 15q11q13 appear to be maternally or paternally imprinted during gametogenesis such that copies from each parent are required for normal development. Deletions from parents of opposite sex manifest clinically distinct syndromes and additional copies of genes from one parent do not balance missing genes from the other parent. Asynchronous replication of maternal and paternal proximal 15q [Izumikawa et al., 1991] may be a consequence of imprinting. Very little is known of its molecular basis. Studies in mice suggest an association between methylation and imprinting in transgenes [Swain et al., 1987; Reik et al., 1987]. Recent evidence suggests that the genetic imprint does not extend over large regions [Barlow et al., 1991] and that genes which are tightly linked can be imprinted in opposite directions [Bartolomei et al., 1991; DeChiara et al., 1991]. A number of expressed chromosome 15q11q13-specific sequences map close to the p (pink-eyed dilution) locus on mouse chromosome 7 [Nicholls, 1989; Chaillet et al., 1991, Wagstaff et al., 1991a], a region for which there is no evidence as yet for imprinting.

The minimum estimated size of chromosome region 15q11q13 is 5 Mb and it may contain more than 100 genes. While several expressed sequences have been found in q11q13, only recently a first gene of known function was localized to this region [Wagstaff et al., 1991b]. This gene encodes a receptor subunit for gamma aminobutyric acid (GABA) which is the main inhibitory neurotransmitter in the mammalian brain. This $GABA_A$ receptor β3 subunit gene (GABRB3) is deleted in AS and PWS patients with interstitial cytogenetic deletions but is not deleted in at least one PWS patient with an unbalanced translocation [Wagstaff et al., 1991b]. It is deleted in the family with a maternally inherited submicroscopic deletion and 3 AS children [Hamabe et al., 1991b; Saitoh et al., 1992]. Thus, GABRB3 has not been excluded from the AS critical region and may be responsible for some of the clinical manifestations of AS.

Although cytogenetically visible interstitial deletions in AS and PWS are similar in size when tested with DNA probes, the critical regions of deletion for the 2 syndromes are being refined through the analysis of patients with unbalanced translocations and submicroscopic deletions [Wagstaff et al., 1991b; Knoll and Lalande, unpublished data]. Current evidence suggests that the critical regions for the 2 syndromes are distinct. More precise definition of the critical regions, as well as identification of genes within these regions, should lead to an understanding of the genes and regulatory elements responsible for the pathogenesis of AS and PWS.

ADDENDUM

We have locallized a second gamma aminobutyric acid receptor subunit gene, α5 (GABRA5) to chromosome 15q11q13. It is deleted in AS and PWS patients with interstitial cytogenetic deletions but is intact in both an AS patient and a PWS patient with unbalanced translocations and smaller distal extents of deletion.

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REFERENCES

Angelman H (1965): "Puppet" children: A report on three cases. Dev Med Child Neurol 7:681–683.

Barlow DP, Stoger R, Herrmann BG, Saito K, Scheifer N (1991): The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the *Tme* locus. Nature 349:84–87.

Bartolomei MS, Semel S, Tilghman SM (1991): Parental imprinting of the mouse H19 gene. Nature 351:153–155.

Buiting K, Neumann M, Ludecke H-J, Senger G, Claussen U, Antich J, Passarge E, Horsthemke B (1990): Microdissection of the Prader-Willi syndrome chromosome region and identification of potential gene sequences. Genomics 6:521–527.

Butler MG (1989): Hypopigmentation: A common feature of Prader-Labhart-Willi syndrome. Am J Hum Genet 45:140–146.

Butler MG, Meaney FJ, Palmer CG (1986): Clinical and cytogenetic survey of 39 individuals with Prader-Labhart-Willi syndrome. Am J Med Genet 23:793–809.

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

Chaillet JR, Knoll JHM, Horsthemke B, Lalande M (1991): The syntenic relationship of the critical deletion region for the Prader-Willi/Angelman syndromes and proximal mouse chromosome 7. Genomics 11:773–776.

DeChiara TM, Robertson EJ, Efstratiadis A (1991): Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64:849–859.

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, 6964.

Gregory CA, Kirkilionis AJ, Greenberg CR, Chudley AE, Hamerton JL (1990): Detection of molecular rearrangements in Prader-Willi syndrome patients by using genomic probes recognizing four loci within the PWCR. Am J Med Genet 35:536–545.

Hamabe J, Fukushima Y, Harada N, Abe K, Matsuo N, Nagai T, Yoshioka A, Tonoki H, Tsukino R, Niikawa N (1991a): Molecular study of the Prader-Willi syndrome: Deletion, RFLP, and phenotype analyses of 50 patients. Am J Med Genet 41:54–63.

Hamabe J, Kuroki Y, Imaizumi K, Sugimoto T, Fukushima Y, Yamaguchi A, Izumikawa Y, Niikawa N (1991b): DNA deletion and its parental origin in Angelman syndrome patients. Am J Med Genet 41:64–68.

Harnden DG, Klinger HP (eds) (1985): "An International System for Human Cytogenetic Nomenclature." New York: S. Karger.

Hultén M, Armstrong S, Challinor P, Gould C, Hardy G, Leedham P, Lee T, McKeown C (1991): Genomic imprinting in an Angelman and Prader-Willi translocation family. Lancet 338:638–639.

Izumikawa Y, Naritomi K, Hirayama K (1991): Replication asynchrony between homologs 15q11.2: Cytogenetic evidence for genomic imprinting. Hum Genet 87:1–5.

Kaplan LC, Wharton R, Elias E, Mandell F, Donlon T, Latt SA (1987): Clinical heterogeneity associated with deletions in the long arm of chromosome 15: Report of 3 new cases and their possible genetic significance. Am J Med Genet 28:45–53.

Knoll JHM, Glatt KA, Nicholls RD, Malcolm S, Lalande M (1991): Chromosome 15 uniparental disomy is not frequent in Angelman syndrome 48:16–21.

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 47:149–155.

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

Lawrence JB, Singer RH, McNeil JA (1990): Interphase and metaphase resolution of different distances within the human dystrophin gene. Science 249:928–932.

Ledbetter DH, Mascarello JT, Riccardi VM, Harper VD, Airhart SD, Strobel RJ (1982): Chromosome 15 abnormalities and the Prader-Willi syndrome: A follow-up report of 40 cases. Am J Hum Genet 34:278–285.

Ledbetter DH, Riccardi VM, Airhart SD, Strobel RJ, Keenan BS, Crawford JD (1981): Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. N Engl J Med 304:325–329.

Lubinsky M, Zellweger H, Greenswag L, Larson G, Mansmann I, Ledbetter D (1987): Familial Prader-Willi syndrome with normal chromosomes. Am J Med Genet 28:37–43.

Magenis RE, Brown MG, Lacy DA, Budden S, LaFranchi S (1987): Is Angelman syndrome an alternate result of del(15)(q11q13)? Am J Med Genet 28:829–838.

Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown MG, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J, Lacy D, LaFranchi S, Lahr M, Macfarlane J, Williams CPS (1990): Comparison of the 15q deletions in Prader-Willi and Angelman syndromes: Specific regions, extent of deletions, parental origin, and clinical consequences. Am J Med Genet 35:333–349.

Malcolm S, Clayton-Smith J, Nichols M, Robb S, Webb T, Armour T, Jeffreys AJ, Pembrey ME (1991): Uniparental disomy in Angelman's syndrome. Lancet 337:694–697.

Mattei MG, Souiah N, Mattei JF (1984): Chromosome 15 anomalies and the Prader-Willi syndrome: Cytogenetic analysis. Hum Genet 66:313–334.

Miller OJ, Schnedl W, Allen J, Erlanger BF (1974): 5-Methylcytosine localised in mammalian constitutive heterochromatin. Nature 251:636–637.

Nicholls RD (1989): A cDNA from proximal human chromosome 15q maps near Mtv-1 on mouse chromosome 7. Mouse Newletter 84:87–88.

Nicholls RD, Knoll JH, Glatt K, Hersh JH, Brewster TD, Graham JM Jr, Wurster-Hill D, Wharton R, Latt SA (1989a): Restriction fragment length polymorphisms within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Am J Med Genet 33:66–77.

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

Niikawa N, Ishikiriyama S (1985): Clinical and cytogenetic studies of the Prader-Willi syndrome: Evidence of phenotype-karyotype correlation. Hum Genet 69:22–27.

Okamoto E, Miller DA, Erlanger BF, Miller OJ (1981): Polymorphism of 5-methylcytosine-rich DNA in human acrocentric chromosomes. Hum Genet 58:225–259.

Pembrey M, Fennell SJ, Van Den Gerghe J, Fiechett M, Summers D, Butler L, Clarke C, Griffiths M, Thompson E, Super M, Baraitser M (1989): The association of Angelman's syndrome with deletions within 15q11-13. J Med Genet 26:73–77.

Prader A, Labhart A, Willi H (1956): Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchismus, und Oligophrenie nach Myotnieartigem Zustand in Neugeborenalter. Schweiz Med Wochenschr 86:1260–1261.

Reik W, Collick A, Norris ML, Barton SC, Surani MAH (1987): Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature 328:248–251.

Robinson WP, Bottani A, Yagang X, Balakrishnan J, Binkert F, Machler M, Prader A, Schinzel A (1991): Molecular, cytogenetic and clinical investigations of Prader-Willi syndrome patients. Am J Hum Genet (in press).

Saadallah N, Hultén M (1983): Chiasma distribution, genetic lengths, and recombination fractions: A comparison between chromosomes 15 and 16. J Med Genet 20:290–299.

Saitoh S, Kubota T, Ohta T, Jinno Y, Niikawa N, Sugimoto T, Wagstaff J, Lalande M (1992): Familial Angelman syndrome caused by imprinted submicroscopic deletion encompassing $GABA_A$ receptor β3-subunit gene. Lancet 338:336–367.

Schmid M, Haaf T, Grunert D (1984): 5-Azacytidine-induced undercondensations in human chromosomes. Hum Genet 67:257–263.

Schweizer D, Ambros P, Andrle M (1978): Modification of DAPI banding on human chromosomes by prestaining with a DNA-binding oligopeptide antibiotic, distamycin A. Exp Cell Res 111:327–332.

Smeets DFC, Merkz GFM, Hopman AHM (1991): Frequent occurrence of translocations of the short arm of chromosome 15 to other D-group chromosomes. Hum Genet 87:45–48.

Swain JL, Stewart TA, Leder P (1987): Paternal legacy determines methylation and expression of an autosomal transgene: A molecular mechanism for parental imprinting. Cell 50:719–727.

Tantravahi U, Nicholls R, Stroh H, Ringer S, Neve RL, Kaplan L, Wharton R, Wurster-Hill D, Graham JM Jr, Cantú ES, Frias JL, Kousseff BG, Latt SA (1989): Quantitative calibration and use of

DNA probes for investigating chromosome abnormalities in the Prader-Willi syndrome. Am J Med Genet 33:78–87.

Tasset D, Hartz JA, Kao FT (1988): Isolation and analysis of DNA markers to human chromosome 15. Am J Hum Genet 42:854–866.

Wagstaff J, Chaillet JR, Lalande M (1991a): The $GABA_A$ receptor β3 subunit gene: Characterization of a human cDNA from chromosome 15q11q13 and mapping to a region of conserved synteny on mouse chromosome 7. Genomics 11:1071–1078.

Wagstaff J, Knoll JHM, Fleming J, Kirkness EF, Martin-Gallardo A, Greenberg F, Graham JM Jr, Menninger J, Ward D, Venter JC, Lalande M (1991b): Localization of the gene encoding the $GABA_A$ receptor B3 subunit to the Angelman/Prader-Willi region of human chromosome 15. Am J Hum Genet 49:330–337.

Wenger SL, Rauch SD, Hanchett JM (1989): Sister chromatid exchange analysis of the 15q11 region in Prader-Willi syndrome patients. Hum Genet 83:111–114.

Wiesner GL, Bendel CM, Olds DP, White JG, Arthur DC, Ball DW, King RA (1987): Hypopigmentation in the Prader-Willi syndrome. Am J Hum Genet 40:431–442.

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

Williams CA, Zori RT, Stone JW, Gray J, Cantú ES, Ostrer H (1990): Maternal origin of 15q11-13 deletions in Angelman syndrome suggests a role for genomic imprinting. Am J Med Genet 35:350–353.