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X-Chromosome Inactivation Patterns in Females With Prader–Willi Syndrome

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Prader–Willi syndrome (PWS) is a complex neurodevelopmental disorder caused by loss of paternally expressed genes from the 15q11-q13 region generally due to a paternally-derived deletion of the 15q11-q13 region or maternal disomy 15 (UPD). Maternal disomy 15 is usually caused by maternal meiosis I non-disjunction associated with advanced maternal age and after fertilization with a normal sperm leading to trisomy 15, a lethal condition unless trisomy rescue occurs with loss of the paternal chromosome 15. To further characterize the pathogenesis of maternal disomy 15 process in PWS, the status of X-chromosome inactivation was calculated to determine whether non-random skewing of X-inactivation is present indicating a small pool of early embryonic cells. We studied X-chromosome inactivation in 25 females with PWS-UPD, 35 with PWS-deletion, and 50 controls (with similar means, medians, and age ranges) using the polymorphic androgen receptor (*AR*) gene assay. A significant positive correlation ($r = 0.5$, $P = 0.01$) was seen between X-chromosome inactivation and age for only the UPD group. Furthermore, a significantly increased level ($P = 0.02$) of extreme X-inactivation skewness (>90%) was detected in our PWS-UPD group (24%) compared to controls (4%). This observation could indicate that trisomy 15 occurred at conceptus with trisomy rescue in early pregnancy leading to extreme skewness in several PWS-UPD subjects. Extreme X-inactivation skewness may also lead to additional risks for X-linked recessive disorders in PWS females with UPD and extreme X-chromosome skewness.

Key words: Prader–Willi syndrome (PWS); UPD; 15q11-q13 deletion; X-inactivation; extreme skewness

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INTRODUCTION

Prader–Willi syndrome (PWS) is characterized by infantile hypotonia, feeding difficulties, hypogonadism, small hands and feet, mental deficiency, behavioral problems, hyperphagia leading to obesity in early childhood, and a particular facial appearance [Cassidy, 1997; Butler and Thompson, 2000]. A deletion of the paternal 15q11-q13 region is found in about 70% of PWS subjects, uniparental maternal disomy 15, or UPD in approximately 25%, and a defect in imprinting in the remaining subjects [Bittel and Butler, 2005]. Clinical differences have been reported between individuals with PWS and the 15q11-q13 deletion or maternal disomy 15. In general, individuals with the deletion have more maladaptive and compulsive behaviors and lower cognition than those with maternal disomy 15 [Butler and Thompson, 2000; Butler et al., 2004]. Recently, a higher incidence of autism spectrum disorders was reported in PWS-UPD subjects compared with deletion PWS subjects [Veltman et al., 2005].

Maternal disomy 15 is thought to occur by maternal meiosis I non-disjunction and associated with advanced maternal age with fertilization by a normal

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sperm leading to trisomy 15 with 47 chromosomes. Loss of the paternal chromosome 15 could then occur in early pregnancy. These cases may initially be detected as mosaic trisomy 15 during routine prenatal diagnostic studies (e.g., chorionic villus sampling) [Christian et al., 1996]. However, maternal disomy 15 could also occur from a disomic 15 oocyte fertilized by a nullisomic 15 sperm producing a normal chromosome complement.

The chromosome 15q11-q13 region is known to contain imprinted DNA sequences that are differentially expressed depending on the parent of origin. Imprinted gene expression is coordinately controlled in *cis* by an imprinting center, which regulates the establishment of parental specific allelic differences in DNA methylation, chromatin structure, and expression [Brannan and Bartolomei, 1999; Nicholls and Knepper, 2001]. The 15q11-q13 region contains about 4 million base pairs of DNA with supporting evidence of at least 30 presumed genes in the region [Bittel et al., 2003; Chai et al., 2003]. However, no single gene has as yet been conclusively shown to account for the PWS phenotype. It is unclear how changes in gene expression resulting from both loss of imprinted genes and reduced expression of non-imprinted genes lead to the clinical findings associated with PWS. Although the chromosome 15q11-q13 region is involved in a deletion process in the majority of PWS subjects, maternal disomy 15 accounts for a significant subset which could involve genes outside of this chromosome region.

X-chromosome inactivation occurs early in embryonic development of somatic cells in human females to achieve gene dosage compensation with males [Lyon, 1961]. This process is thought to take place within 7–10 days after fertilization when the embryonic cell mass contains no more than a few dozen cells [Avner and Heard, 2001]. Therefore, one of the two X-chromosomes is inactivated in each female cell generally at random which then results in a similar number of active X-chromosome genes in both male and female cells. The X-inactivation is a complex process which can be altered by various processes but requires three main steps: initiation, spreading, and maintenance [Willard, 1995; Penny et al., 1996]. During the initiation step, one of the two X-chromosomes is selected to remain activated and requires the presence in *cis* of the X-inactivation center (XIC) [Russell, 1963; Brown et al., 1991; Migeon, 2006].

The human androgen receptor (*AR*) gene located at Xq11.2 contains a highly polymorphic in-frame CAG gene codon repeat encoding 11–31 glycine residues in exon 1. X-inactivation patterns can be assessed using the *AR* gene in females informative at the CAG repeat following DNA digestion with methyl-sensitive restriction enzymes (e.g., *Hpa*II) and PCR amplification of the polymorphic *AR* gene. X-chromosome skewness (i.e., one X-chromosome may be more or less active compared with the second X-in somatic cells) is assigned at an arbitrary ratio of highly skewed (e.g., >80%:20%) or extremely skewed (e.g., >90%:10%) [Harris et al., 1992; Sangha et al., 1999; Maier et al., 2002; Talebizadeh et al., 2005]. In healthy females, X-chromosome inactivation is considered to follow a Gaussian or bell-shaped distribution with highly skewed patterns being uncommon events [Migeon, 1998].

Since about 2% of pregnancies detected by chorionic villus sampling are associated with confined placental mosaicism [Ledbetter et al., 1992; Wang et al., 1993], it may be a significant contributor to both skewed X-inactivation observed in some newborns and expression of X-linked recessive diseases in females. Extreme skewed X-inactivation is also seen in female carriers of dyskeratosis congenita [Sangha et al., 1999] and in women with recurrent spontaneous abortions [Sun and Baumer, 1999; Bretherick et al., 2005] involving placental trisomy mosaicism [Beever et al., 2003]. Non-random X-inactivation was also suggested to explain reduced penetrance in carrier females with the fragile X-gene mutation [Naumova et al., 1996], levels of FMR protein in blood, and the fragile X-phenotype from fully mutated female carriers [Martinez et al., 2005]. Skewed X-inactivation has also been reported in lymphocytes from females with Rett syndrome, a severely disabling neurologic disorder due to *MECP2* gene mutations located at Xq28 [Krepischi et al., 1998]. X-chromosome inactivation patterns were also reported in individuals with Russell–Silver syndrome (RSS) and their parents [Beever et al., 2003]. Interestingly, about 10% of RSS cases are found to have maternal disomy 7 but no increase in X-chromosome inactivation skewness was seen in the RSS cases. However, an increase of skewness was found in the mothers of children with RSS. In addition, we reported that non-random X-inactivation occurs in females with classical autism which may indicate X-linked genes playing a role in the reported higher male to female ratio seen in this disorder [Talebizadeh et al., 2005].

Extreme X-chromosome skewing (>90%) in PWS females with maternal disomy 15 may indicate a small pool of embryonic progenitor cells originating from rescue of trisomy 15 and could further suggest that those females could be at risk for X-linked recessive disorders along with PWS. Previously, a higher prevalence of X-inactivation skewness was suggested for PWS females with UPD [Lau et al., 1997; Robinson et al., 2000]. Therefore, to determine the frequency and level of X-chromosome inactivation skewness in Prader–Willi syndrome with either genetic subtype (15q deletion and maternal disomy 15) and to determine age effects (if any) compared with control females, we performed X-inactivation studies on genomic DNA from peripheral blood samples from 110 females (PWS and control).

SUBJECTS AND METHODS

Subjects

One hundred ten females were recruited in the clinical setting and agreed to participate in the study by signing informed consent forms approved by the local Institutional Review Board. They consisted of 60 with Prader–Willi syndrome (35 with 15q11-q13 deletion with an average (± SD) age of 16.2 ± 10.2 years, median age of 16 years, and range of 0.5–39 years and 25 with maternal disomy 15 (UPD) with an average (± SD) age of 19.1 ± 9 years, median age of 20, and range of 0.1–34 years) and 50 similarly aged healthy unrelated females without Prader–Willi syndrome with an average (±SD) age of 18.3 ± 10.3 years, median age of 14.5 years, and range of 0.5–39 years. Prader–Willi syndrome diagnosis was confirmed by methylation DNA testing and genetic subtypes determined by chromosome analysis with FISH using probes from the 15q11-q13 region for identification of the 15q11-q13 deletion and microsatellite DNA studies for identification of maternal disomy 15 [Butler et al., 2004].

Description of X-chromosome inactivation assay

In order to evaluate X-inactivation, genomic DNA was extracted from peripheral blood and amplified with polymerase chain reaction (PCR) in the presence of forward and reverse primers for the polymorphic *AR* gene [Talebizadeh et al., 2005]. The polymorphic CAG repeat size is determined by capillary electrophoresis using an ABI 3100 DNA sequencer (Foster City, CA). Subsequently, 200 ng of genomic DNA was digested with the methyl sensitive *Hpa*II restriction enzyme as described elsewhere [Allen et al., 1992]. The 5′ end of the forward primer was fluorescently labeled with 6-FAM (6-carboxy-fluorescein) and the resulting PCR fragments analyzed by capillary electrophoresis following established protocols [Karasawa et al., 2001; Villard et al., 2001]. To account for preferential allele amplification, values for the digested DNA were normalized with those for the undigested DNA for each subject. Thus, X-chromosome inactivation was calculated by applying the following formula: [(phd1/phu1)/(phd1/phu1) + (phd2/phu2)] where phd1 = peak height of 1st allele (digested DNA); phd2 = peak height of 2nd allele (digested DNA); phu1 = peak height of 1st allele (undigested DNA); phu2 = peak height of 2nd allele (undigested DNA) as described elsewhere (Lau et al., 1997). Repeated experiments were performed to confirm complete digestion of genomic DNA and to ensure correct assessment of the ratio of an active (unmethylated) versus an inactive (methylated) X-chromosome as described previously [Talebizadeh et al., 2005].

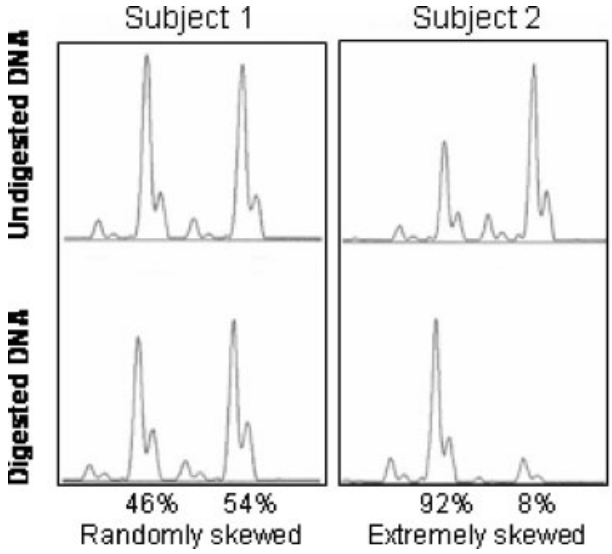

FIG. 1. X-inactivation analysis by genotyping of a polymorphic region in the *AR* gene (CAG repeat) using methyl sensitive restriction enzyme (*HpaII*) method. Genotyping of undigested genomic DNA (top) and *HpaII* digested DNA (bottom) are shown. The peak representing the DNA from the active (unmethylated) X-chromosome allele would be digested by the methyl sensitive enzyme and reduced in size. If non-random X-inactivation skewness is present the peak height would differ after digestion between the two alleles representing each X-chromosome (methylated-inactive and unmethylated-active). Examples of randomly and extremely skewed (>90%) X-inactivation are shown.

Extreme X-inactivation skewness was identified when the calculated ratio was >90% for one *AR* gene allele in digested DNA (see Fig. 1).

RESULTS

Using the X-inactivation assay described above, we studied X-chromosome skewness in 110 females (PWS and control) with an age range of birth to 39 years of age and informative for the CAG repeat of the polymorphic *AR* gene (see Table I). A greater percentage of nonrandom X-inactivation skewness (extremely skewed >90%:10%) was seen in the maternal disomy 15 group (6 of 25 or 24%) compared with the control females (2 of 50 or 4%) ($P = 0.02$; Chi-square test) but not with those with 15q11-q13 deletion (3 of 35 or 9%) ($P = 0.30$; Chi-square test) (see Fig. 2). When comparing all three subgroups (PWS-UPD, PWS-del and control females) with the Chi-square test after Bonferroni adjustment for three pairwise comparisons, a significant *P*-value of 0.02 was calculated. Pearson correlations were calculated between X-inactivation data and age for the three subject groups (PWS-UPD; PWS-del; control females) and found to be significant ($r = 0.5$, $P = 0.01$) for only the PWS-UPD subjects.

DISCUSSION

X-inactivation has previously been studied in subjects with PWS-UPD and a higher prevalence of

TABLE I. Age and X-Inactivation Data for the Prader–Willi Syndrome (PWS) and Control Females

PWS-UPD (N = 25)		PWS-deletion (N = 35)		Control (N = 50)	
Age (yr)	X-inactivation skewness	Age (yr)	X-inactivation skewness	Age (yr)	X-inactivation skewness
0.1	57%: 43%	0.5	58%: 42%	0.5	36%: 64%
3	25%: 75%	2	75%: 25%	5	33%: 67%
5	50%: 50%	2	46%: 54%	6	89%: 11%
7	37%: 63%	3	65%: 35%	7	70%: 30%
13	50%: 50%	3	82%: 18%	8	39%: 61%
13	81%: 19%	3	46%: 54%	8	68%: 32%
14	59%: 41%	4	57%: 43%	9	87%: 13%
15	50%: 50%	6	66%: 34%	9	28%: 72%
15	54%: 46%	8	60%: 40%	9	57%: 43%
18	60%: 40%	9	20%: 80%	10	61%: 39%
18	36%: 64%	10	43%: 57%	10	45%: 55%
19	***96%: 4%***	12	18%: 82%	10	61%: 49%
20	***95%: 5%***	13	63%: 37%	10	60%: 40%
21	74%: 26%	13	31%: 69%	10	56%: 44%
22	***92%: 8%***	13	45%: 55%	11	54%: 46%
23	53%: 47%	15	22%: 78%	11	38%: 62%
23	71%: 29%	15	49%: 51%	11	63%: 37%
26	49%: 51%	16	50%: 50%	12	23%: 77%
26	30%: 70%	19	61%: 39%	12	***7%: 93%***
27	18%: 82%	19	12%: 88%	12	24%: 76%
27	***3%: 97%***	19	***9%: 91%***	13	79%: 21%
29	69%: 31%	19	35%: 65%	13	50%: 50%
30	***95%: 5%***	20	60%: 40%	13	56%: 44%
30	***5%: 95%***	20	50%: 50%	13	16%: 84%
34	88%: 12%	20	***4%: 96%***	14	34%: 66%
		21	57%: 43%	15	53%: 47%
		22	60%: 40%	15	63%: 37%
		23	70%: 30%	18	71%: 29%
		23	67%: 33%	18	***7%: 93%***
		28	47%: 53%	19	70%: 30%
		28	71%: 29%	22	59%: 41%
		30	50%: 50%	22	30%: 70%
		30	43%: 57%	22	76%: 24%
		39	***9%: 91%***	23	67%: 33%
		39	13%: 87%	23	56%: 44%
				23	35%: 65%
				25	31%: 69%
				25	73%: 27%
				25	49%: 51%
				26	42%: 58%
				26	38%: 62%
				26	68%: 32%
				30	27%: 73%
				34	47%: 53%
				37	55%: 45%
				37	18%: 82%
				38	60%: 40%
				39	56%: 44%
				39	12%: 88%
				39	45%: 50%

Age (yr)	UPD	Deletion	Control
Mean	19.1	16.2	18.3
St. Deviation	9.0	10.2	10.3
Median	20	16	14.5
Minimum	0.1	0.5	0.5
Maximum	34	39	39

Extreme X-inactivation skewness is shown in bold italics.

X-inactivation skewness was found in this group of PWS females compared with controls [Lau et al., 1997; Robinson et al., 2000]. This finding has not been replicated in other studies on PWS females. Thus, there are few reports of X-inactivation data in PWS females particularly in different genetic subtypes (deletion vs. UPD). However, X-inactivation studies have been reported in other neurodevelopmental conditions. For example, a significantly higher percentage of X-inactivation skewness was reported in X-linked mental retardation carriers [Plenge et al., 2002] and a high concordance of skewing of X-inactivation observed between mothers and daughters in families with dystrophinopathies [Azofeifa et al., 1995; Yoshioka et al., 1998]. In addition, Villard et al. [2001] described a totally skewed pattern of X-inactivation in four familial cases of Rett syndrome without the *MECP2* gene mutation. Female carriers of X-linked adrenoleukodystrophy (X-ALD) were also more susceptible to X chromosome inactivation

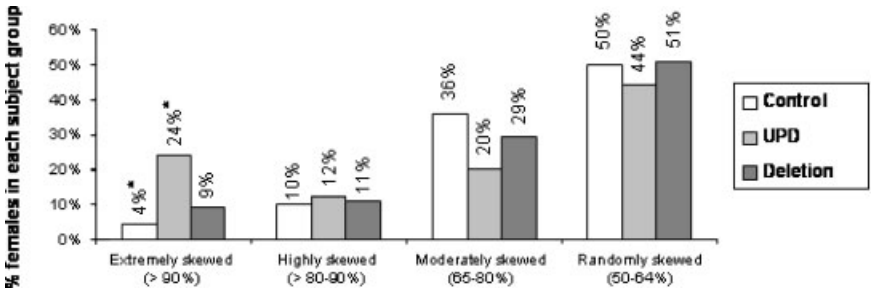

FIG. 2. Distribution of patterns of X-inactivation in 50 controls, 25 PWS-UPD, and 35 PWS-deletion females. X-inactivation patterns were divided into four categories (i.e., extremely, highly, moderately, and randomly skewed). A significantly greater percentage (Chi-square test; $P < 0.05$) of extremely skewed X-inactivation (>90%) was detected in the PWS-UPD group (24%) compared with controls (4%) shown with asterisks.

skewness [Maier et al., 2002] with an equal proportion of moderate and highly skewed findings observed in X-ALD female carriers. We also reported X-chromosome skewness in females with autism [Talebizadeh et al., 2005]. Furthermore, extreme skewing of X-inactivation was observed in fetuses and newborns associated with confined placental mosaicism (CPM) of an autosomal trisomy [Lau et al., 1997; Beever et al., 2003]. This non-random X-inactivation could conceivably result from a reduction in the size of the early embryonic cell pool due to poor growth or selection against trisomic cells. Overall a higher degree of skewing (>90%) was observed in CPM due to a meiotic origin of the trisomic cell line in the placenta which would also affect the embryonic progenitors compared to CPM thought due to a somatic cause arising in either the trophoblast or chorionic lineages confined to the placenta after differentiation [Lau et al., 1997].

Alternatively if maternal disomy 15 was due to fertilization of a disomic 15 oocyte with a nullisomic 15 sperm, allowing for a 46 chromosome complement, then no increased X-chromosome skewing would be anticipated. Our study of X-inactivation in a relatively large cohort of females with PWS of both genetic subtypes (deletion and UPD) is in agreement with previous studies and further supports the observation of a higher degree of X-inactivation skewness in females with PWS and maternal disomy 15 [Lau et al., 1997; Robinson et al., 2000].

We found that 24% of the PWS-UPD females showed extreme X-chromosome inactivation (>90%) compared with 4% in our control females. In general, only about 1% of females reported in the literature have patterns as skewed as 95% or greater [Allen et al., 1992; Naumova et al., 1996] which is in agreement with our findings in either the PWS-deletion (1 out of 35) or control subjects (0 out of 50). However, 20% (5 out of 25) of the PWS-UPD females in our study exhibited patterns of skewness of ≥95%.

Possible explanations for the observed X-chromosome skewness in females with PWS and maternal disomy 15 may include selective cell death after initial random X-inactivation (e.g., carriers of X-autosome translocations, lymphocytes of carriers of X-linked immunodeficiency disease) but probably unlikely in the peripheral blood of females with PWS. A second possibility for the X-chromosome skewness may be selective ascertainment of individuals from the tail of a random distribution of inactivation because of an unusual or unexpected phenotype. Examples of this phenomenon would include female carriers of Duchenne muscular dystrophy manifesting the disease state. However, the more likely explanation would be trisomy rescue in females with trisomy 15 cells at conception. The loss of cells results in a smaller population of viable cells to proliferate leading to possible non-random X-inactivation observed in females with PWS and maternal disomy 15.

X-chromosome inactivation is a complex, multiprocess phenomenon which involves several epigenetic factors such as: DNA methylation, X-chromosome reactivation, parental origin effect (imprinting), possible factors influencing X-inactivation skewness and loss of cells in fetal development which may mimic clonal evolution of cells particularly in PWS individuals with non-random skewness and UPD. While X-inactivation is a stable process in human somatic cells, deviation from this general rule does exist. For example, the human placenta differs from other somatic tissues and is capable to reverse X-inactivation [Migeon et al., 2005]. Additionally, X-inactivation variation has been reported in human embryonic stem cell lines [Hoffman et al., 2005].

X-inactivation patterns in females have shown variation with age [Busque et al., 1996] particularly increased skewness with advancing age. Acquired skewness occurs with aging in control females and is reported in 38% of females over the age of 60 and as low as 9% in neonates (using *AR* gene allele ratios ≥3:1) [Busque et al., 1996]. Therefore, previous X-inactivation studies in control females suggest that the increase in skewness occurs after 50–60 of age and may not be detected in females with a younger age range [Sharp et al., 2000; Sandovici et al., 2004; Kristiansen et al., 2005]. This is in agreement with the lack of significant correlations with X-inactivation data and age in our controls or PWS-deletion

females. However, we found a significant correlation with X-chromosome inactivation and age in the PWS-UPD group at a relatively young age range (0.1–34 years). Whether the acquired skewing in older control females is a consequence of stem cell depletion, true clonal hematopoiesis, growth advantage conferred by parental specific X-chromosome or other causes is not known [Busque et al., 1996]. The presence of such a correlation with age in the PWS-UPD females may need further investigation with a larger sample size including a wider age range as well as longitudinal studies over several decades to measure the impact of age.

In summary, our study supports the presence of significantly higher prevalence of extreme X-chromosome skewness in PWS-UPD females compared with controls indicating the possible existence of trisomy 15 at conception with loss of embryonic cells in several PWS-UPD females. Through trisomy rescue events early in embryo development, a small number of embryonic cells could have a selective advantage for cell proliferation due to a normal chromosome complement and maternal disomy 15 but with extreme X-skewness. It is not clear if the X-inactivation pattern in blood correlates with other tissues such as brain. While our study indicates significantly higher X-inactivation skewness in PWS-UPD compared with PWS-deletion females using peripheral blood DNA, this observation should also be evaluated in other tissues. In those PWS females with UPD and extreme X-chromosome skewness, there is a possibility that X-linked recessive conditions could be present along with PWS. Future studies to include a larger sample size and correlations between X-inactivation skewness and clinical features in PWS-UPD females maybe helpful in understanding the relationship (if any) between extreme X-chromosome skewness and clinical presentation.

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REFERENCES

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. 1992. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen- receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229–1239.

Avner P, Heard E. 2001 . X-chromosome inactivation: Counting, choice and initiation. Nat Rev Genet 2 :59–67.

Azofeifa J, Voit T, Hubner C, Cremer M. 1995. X-chromosome methylation in manifesting and healthy carries of dystrophinopathies: Concordance of activation ratios among first degree female relatives and skewed inactivation as cause of the affected phenotypes. Hum Genet 96:167–176.

Beever CL, Stephenson MD, Penaherrera MS, Jiang RH, Kalousek DK, Hayden M, Field L, Brown CJ, Robinson WP. 2003. Skewed X-chromosome inactivation is associated with trisomy in women ascertained on the basis of recurrent spontaneous abortion or chromosomally abnormal pregnancies. Am J Hum Genet 72:399–407.

Bittel DC, Butler MG. 2005. Prader–Willi syndrome: Clinical genetics, cytogenetics, and molecular biology. Expert Rev Mol Med 7:1–20.

Bittel DC, Kibiryeva N, Talebizadeh Z, Butler MG. 2003. Microarray analysis of gene/transcript expression in Prader–Willi syndrome: Deletion versus UPD. J Med Genet 40:568–574.

Brannan CI, Bartolomei MS. 1999. Mechanisms of genomic imprinting. Curr Opin Genet Dev 9:164–170.

Bretherick K, Gair J, Robinson WP. 2005. The association of skewed X chromosome inactivation with aneuploidy in humans. Cytogenet Genome Res 111:260–265.

Brown CJ, Lafreniere RG, Powers VE, Sebastio G, Ballabio A, Pettigrew AL, Ledbetter DH, Levy E, Craig IW, Willard HF. 1991. Localization of the X-inactivation center on the human X chromosome in Xq13. Nature 349:82–84.

Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, Maragh M, Gilliland DG. 1996. Nonrandom X-inactivation patterns in normal females: Lyonization ratios vary with age. Blood 88: 59–65.

Butler MG, Thompson T. 2000. Prader–Willi syndrome: Clinical and genetic findings. Endocrinologist 10:3S–16S.

Butler MG, Bittel DC, Kibiryeva N, Talebizadeh Z, Thompson T. 2004. Behavioral differences among subjects with Prader–Willi syndrome and type I or type II deletion and maternal disomy. Pediatrics 113:565–573.

Cassidy SB. 1997. Syndrome of the month: Prader–Willi syndrome. J Med Genet 34:917–923.

Chai JH, Locke DP, Greally JM, Knoll JH, Ohta T, Dunai J, Yavor A, Eichler EE, Nicholls RD. 2003. Identification of four highly conserved genes between breakpoint hotspots BP1 and BP2 of the Prader–Willi/Angelman syndromes deletion region that have undergone evolutionary transposition mediated by flanking duplicons. Am J Hum Genet 73:898–925.

Christian SL, Smith AC, Macha M, Black SH, Elder FF, Johnson JM, Resta RG, Surti U, Suslak L, Verp MS, Ledbetter DH. 1996. Prenatal diagnosis of uniparental disomy 15 following trisomy 15 mosaicism. Prenat Diagn 16:323–332.

Harris A, Collins J, Vetrie D, Cole C, Bobrow M. 1992. X-inactivation as a mechanism of selection against lethal alleles: Further investigation of incontinentia pigmenti and X linked lymphoproliferative disease. J Med Genet 29:608–614.

Hoffman LM, Hall L, Batten JL, Young H, Pardasani D, Baetge EE, Lawrence J, Carpenter MK. 2005. X-inactivation status varies in human embryonic stem cell lines. Stem Cells 23:1468–1478.

Karasawa M, Tsukamoto N, Yamane A, Okamoto K, Maehara T, Yokohama A, Nojima Y, Omine M. 2001. Analysis of the distribution of CAG repeats and X-chromosome inactivation status of HUMARA gene in healthy female subjects using improved fluorescence-based assay. Int J Hematol 74:281–286.

Krepischi AC, Kok F, Otto PG. 1998. X chromosome inactivation patterns in patients with Rett syndrome. Hum Genet 102:319–321.

Kristiansen M, Knudsen GP, Bathum L, Naumova AK, Sorensen TI, Brix TH, Svendsen AJ, Christensen K, Kyvik KO, Orstavik KH. 2005. Twin study of genetic and aging effects on X chromosome inactivation. Eur J Hum Genet 13:599–606.

Lau AW, Brown CJ, Peñaherrea M, Langlois S. Kalousek DK, Robinson WP. 1997. Skewed X-chromosome inactivation is common in fetuses or newborns associated with confined placental mosaicism. Am J Hum Genet 61:1353–1361.

Ledbetter DH, Zachary JM, Simpson JL, Golbus MS, Pergament E, Jackson L, Mahoney MJ, Desnick RJ, Schulman J, Copeland KL, et al. 1992. Cytogenetic results from the U.S. Collaborative Study on CVS. Prenat Diagn 12:317–345.

Lyon MF. 1961. Gene action in the X-chromosome of the mouse (Mus musculus L.). Naturwissenschaften 190:372–373.

Maier EM, Kammerer S, Muntau AC, Wichers M, Braun A, Roscher AA. 2002. Symptoms in carriers of adronoleukodystrophy relate to skewed X-inactivation. Ann Neurol 52:683–688.

Martinez R, Bonilla-Henao V, Jimenez A, Lucas M, Vega C, Ramos I, Sobrino F, Pintado E. 2005. Skewed X-inactivation of the normal allele in fully mutated female carriers determines the levels of FMRP in blood and the fragile X phenotype. Mol Diagn 9:157–162.

Migeon BR. 1998. Non-random X chromosome inactivation in mammalian cells. Cytogenet Cell Genet 80:142–148.

Migeon BR. 2006. The role of X inactivation and cellular mosaicism in women's health and sex-specific diseases. JAMA 295:1428–1433.

Migeon BR, Axelman J, Jeppesen P. 2005. Differential X reactivation in human placental cells: Implications for reversal of X inactivation. Am J Hum Genet 77:355–364.

Naumova AK, Plenge RM, Bird LM, Leppert M, Morgan K, Willard HF, Sapienza C. 1996. Heritability of X chromosome inactivation phenotype in a large family. Am J Hum Genet 58:1111–1119.

Nicholls RD, Knepper JL. 2001. Genome organization, function, and imprinting in Prader–Willi and Angelman syndromes. Annu Rev Genomics Hum Genet 2:153–175.

Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. 1996. Requirement for XIST in X chromosome inactivation. Nature 379:131–137.

Plenge RM, Stevenson RA, Lubs HA, Schwartz CE, Willard HF. 2002. Skewed X-chromosome inactivation is a common feature of X-linked mental retardation disorders. Am J Hum Genet 71:168–173.

Robinson WP, Christian SL, Kuchinka BD, Penaherrera MS, Das S, Schuffenhauer S, Malcolm S, Schinzel AA, Hassold TJ, Ledbetter DH. 2000. Somatic segregation errors predominantly contribute to the gain or loss of a paternal chromosome leading to uniparental disomy for chromosome 15. Clin Genet 57:349–358.

Russell LB. 1963. Mammalian X-chromosome action: Inactivation limited in spread and region of origin. Science 140:976–978.

Sandovici I, Naumova AK, Leppert M, Linares Y, Sapienza C. 2004. A longitudinal study of X-inactivation ratio in human females. Hum Genet 115:387–392.

Sangha KK, Stephenson MD, Brown CJ, Robinson WP. 1999. Extremely skewed X-chromosome inactivation is increased in women with recurrent spontaneous abortion. Am J Hum Genet 65:913–917.

Sharp A, Robinson D, Jacobs P. 2000. Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. Hum Genet 107:343–349.

Sun YJ, Baumer A. 1999. Nonrandom X inactivation and selection of fragile X full mutation in fetal fibroblasts. Am J Med Genet 86:162–164.

Talebizadeh Z, Bittel DC, Veatch OJ, Kibiryeva N, Butler MG. 2005. Brief report: Non-random X chromosome inactivation in females with autism. J Autism Dev Disord 35:675–681.

Veltman MW, Craig EE, Bolton PF. 2005. Autism spectrum disorders in Prader–Willi and Angelman syndromes: A systematic review. Psychiatr Genet 15:243–254.

Villard L, Levy N, Xiang F, Kpebe A, Labelle V, Chevillard C, Zhang Z, Schwartz CE, Tardieu M, Chelly J, Anvret M, Fontes M. 2001. Segregation of a totally skewed pattern of X chromosome inactivation in four familial cases of Rett syndrome without MECP2 mutation: Implications for the disease. J Med Genet 38:435–442.

Wang BB. Rubin CH, Williams JD. 1993. Mosaicism in chorionic villus sampling: An analysis of incidence and chromosomes involved in 2612 consecutive cases. Prenat Diagn 13:179–190.

Willard HF. 1995. The sex chromosome and X-chromosome inactivation. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill. p 717–737.

Yoshioka M, Yorifuji T, Mituyoshi I. 1998. Skewed X inactivation in manifesting carriers of Duchenne muscular dystrophy. Clin Genet 53:102–107.