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Molecular genetic classification in Prader-Willi syndrome: a multisite cohort study

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Abstract

Background—Prader-Willi syndrome (PWS) is due to errors in genomic imprinting. PWS is recognised as the most common known genetic cause of life-threatening obesity. This report summarises the frequency and further characterises the PWS molecular classes and maternal age effects.

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Contributors

MGB planned the study design, recruited individuals, collected natural histories and biological samples and served as the primary author of the manuscript. SNH performed genetic analysis of individuals, compiled data from the different sites and contributed to the manuscript. WAH performed genetic analysis of individuals and compiled data from the different sites. AMM and RT performed statistical analyses and contributed to the manuscript. VK recruited individuals, collected natural histories and biological samples and served as an author of the manuscript. ED and DJD planned the study design, recruited individuals, collected natural histories and biological samples and served as an author of the manuscript. JAG and JLM compiled data and contributed to the manuscript. SJK performed genetic analysis of individuals and compiled data. NW recruited individuals, collected natural histories and biological samples and contributed to the manuscript.

Competing interests None declared.

Patient consent obtained.

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Methods—High-resolution microarrays, comprehensive chromosome 15 genotyping and methylation-specific multiplex ligation probe amplification were used to describe and further characterise molecular classes of maternal disomy 15 (UPD15) considering maternal age.

Results—We summarised genetic data from 510 individuals with PWS and 303 (60%) had the 15q11-q13 deletion; 185 (36%) with UPD15 and 22 (4%) with imprinting defects. We further characterised UPD15 findings into subclasses based on the presence (size, location) or absence of loss of heterozygosity (LOH). Additionally, significantly older mothers (mean age=32.5 years vs 27.7 years) were found in the UPD15 group (n=145) compared with the deletion subtype (n=200).

Conclusions—We report on molecular classes in PWS using advanced genomic technology in the largest cohort to date. LOH patterns in UPD15 may impact the risk of having a second genetic condition if the mother carries a recessive mutant allele in the isodisomic region on chromosome 15. The risk of UPD15 may also increase with maternal age.

Introduction

Prader-Willi syndrome (PWS) is a rare, complex genetic disorder and recognised as the most common syndromic form of obesity reported to affect between 350 000 and 400 000 individuals worldwide involving both sexes equally.¹ The estimated prevalence of PWS ranges between 1 in 10 000–45 000 individuals.²

PWS is a multisystem disorder characterised by severe hypotonia, a poor suck, with failure to thrive, hypogonadism/hypogonitalism, hypothalamic dysfunction with growth and other hormone deficiencies and global developmental delay during infancy. In early childhood, an excessive appetite with obesity, short stature and behaviour problems (eg, compulsions, temper tantrums and skin picking) are present.^{1–5}

PWS results from errors in genomic imprinting with loss of expression of paternal genes in the chromosome 15q11-q13 region usually from a paternal deletion historically quoted at 70%. Maternal disomy 15 (UPD15) in which both chromosome 15s are inherited from the mother is reported in 25%–30%, and the remaining individuals have defects of the imprinting centre, chromosome 15 translocations or inversions.³⁴ There are two types of typical paternal chromosome 15q deletions, 15q11-q13 type I deletion which is about 6 Mb in size involving a proximal chromosome 15 breakpoint (BP1) and 500 kb larger than the typical type II deletion which involves a separate proximal breakpoint (BP2). The region between BP1 and BP2 includes four non-imprinted genes (*NIPA1*, *NIPA2*, *CYFIPI*, *GCP5*) which are intact in the type II deletion but deleted in the type I deletion.⁶ These four genes when deleted (ie, 15q11.2 BP1-BP2) are associated with neurodevelopmental disturbances and developmental delays.⁷⁸ Individuals with a type I deletion are more affected with compulsions and self-injury and perform more poorly on cognitive and behaviour instruments.⁶⁹¹⁰ Atypical deletions occur in 7%–9% of those with deletions and are larger or smaller than the typical type I or type II deletions.⁴¹¹¹² Those with UPD15 have higher verbal IQs but may be more prone to psychosis and autism.⁴⁵ UPD15 may be associated with the generation of homozygous genomic regions of varied length arising from crossing over events in female gametogenesis leading to loss of heterozygosity (LOH) defined as >8 Mb in length.⁵ We further delineated three recognised forms or subclasses of UPD15 based

on the presence or absence of LOH. Specific patterns and frequencies have not been characterised previously or clinical implications addressed in PWS.

Genetic testing is an integral component in the diagnosis of PWS, particularly in infancy before classical syndromic features are present with high specificity. Early diagnosis is vital to ensure intervention during infancy prior to the onset of obesity, hyperphagia, short stature, growth and other hormone deficiencies and behavioural problems.³⁵ Early diagnosis may lower costs for medical care and extended hospitalisations related to the duration of assisted feeding such as gastric tube placement used to treat poor suck reflex and feeding difficulties and complex diagnostic evaluations prior to advanced genetic testing. Recent studies show that commercially insured patients with PWS (at a median age of 10 years) have annual medical costs at 8.8 times greater than unaffected counterparts.¹³ Identifying the molecular class with advanced genetic testing such as high-resolution SNP microarrays allows for a more accurate diagnosis and prognosis.

This study represents the largest survey of PWS molecular classes to date utilising a large multisite cohort of 510 individuals with genetically confirmed PWS recruited at several sites in the USA. By using high-resolution SNP microarrays, methylation-specific multiplex ligation probe amplification (MS-MLPA) and/or chromosome 15 genotyping, we determined the frequency of PWS genetic deletion subtypes and characterised UPD15 subclasses based on LOH patterns.

Subjects and Methods

Subjects

Individuals with genetically confirmed PWS were recruited from five sites directed by internationally known experts for genetic analysis, natural history and genotype-phenotype correlations: 510 individuals with PWS (231 male; 279 female), primarily Caucasians and ranged in age from 0.2 to 62 years. The parental age was collected, specifically on the mother to examine for maternal age differences between PWS genetic subtypes (ie, deletion vs UPD15). Each subject or legal guardian signed the approved human subject's research consent forms prior to enrolment and study.

Genetic testing

Saliva or blood samples were collected for DNA isolation and molecular class identified utilising high-resolution SNP Affymetrix 6.0 microarrays (Santa Clara, CA; figure 1A) or MS-MLPA kits obtained from MRC-Holland (Amsterdam, Netherlands) following established protocols (see figure 1B).¹²¹⁴ Chromosomal microarrays with SNP probes were used to detect UPD15 (heterodisomy, segmental isodisomy, total isodisomy) based on LOH¹⁵ (see figure 1C–E). Genotyping of microsatellite markers from chromosome 15 was undertaken by PCR using subject and parental DNA to identify biparental inheritance and imprinting defects in those with abnormal methylation but no recognised deletion or LOH.

Chromosome deletions are found using high-resolution microarrays when the size is greater than or equal to 100 kb and involve at least 50 DNA markers. Regions of homozygosity are defined as greater than or equal to 3 Mb in size. When these regions are seen on a single

chromosome and greater than or equal to 8 Mb in size or sometimes less (eg, 5 Mb when PWS methylation pattern is present),⁵¹⁴ they represent LOHs which are diagnostic for UPD as seen in PWS with UPD15.

UPD15 is identified and subclass status assigned by the presence (size and location) or absence of LOH regions on chromosome 15. Segmental disomy 15 is identified when one or more LOHs are present on the long arm of chromosome 15. Total isodisomy 15 is identified when the entire length of chromosome 15 (about 80 Mb) demonstrates an LOH.¹⁴ Maternal heterodisomy 15 is indistinguishable using SNP microarrays from normal biparental inheritance. If no LOHs are seen and no deletions (normal copy number) along with an abnormal methylation pattern, then chromosome 15 genotyping and DNA polymorphism studies will be required to show the presence of both 15s from the mother. If genotyping shows normal biparental inheritance, then this patient would have an imprinting defect. The Affymetrix Chromosome Analysis Suite V 1.2.2 software program was used throughout for microarray analysis.

Results

Table 1 shows the PWS genetic findings for 510 individuals with genetically confirmed PWS. For those with complete data, 303 had a 15q11-q13 deletion (60% of cases), 118 (38.9%) with 15q11-q13 type I deletion, 165 (54.5%) with 15q11-q13 type II deletion and 20 (6.6%) had a larger or smaller atypical 15q11-q13 deletion. Of the 185 individuals with UPD15, 104 had the UPD15 subclass established with 31 (29.8%) having maternal heterodisomy 15; 60 (57.7%) with maternal segmental isodisomy 15; and 13 (12.5%) with total maternal isodisomy 15. While 22 (4%) had imprinting defects with 13 (76.5%) having non-deletion epimutations, four (23.9%) had imprinting centre microdeletions and five had imprinting defect status not further established as a microdeletion or epimutation. Significantly older mothers were also found in the UPD15 group ($n = 145$) with an average age of 32.5 years compared with 200 mothers from the deletion group with an average age of 27.7 years (Wilcoxon test; $p < 0.001$), further supporting non-disjunction events in meiosis.

The frequency of the 15q11-q13 deletion was found at a lower rate (60%) than historically quoted and UPD15 at a higher rate (36%). Of the 104 individuals with UPD15, 31 (29.8%) had maternal heterodisomy 15, 59 (57.7%) had segmental isodisomy 15, and 13 (12.5%) had total isodisomy 15. Sixty individuals with segmental isodisomy 15 had a total average LOH size of 25.1 Mb with a range of 5–67.4 Mb and an average size of 16.4 Mb for individual LOHs. Thirty-two individuals had one segment, 25 individuals had two segments and three individuals had three segments. The most common LOH sites were the proximal 15q11-q13 region and distal 15q26 region. The 15q12 and 15q26.1 bands were most often recorded (see figure 1F).

Discussion

The advent of more accurate and reliable genetic technology has changed data interpretation, reported frequencies and identification of molecular genetic classes in PWS.¹⁶ An advanced maternal age, which is becoming more prevalent in Westernised societies, may also impact

the frequency of PWS genetics classes.¹⁶ For example, Cassidy *et al*¹⁷ suggested a relationship between UPD15 and advanced maternal age as ascribed to non-disjunction during meiosis forming a trisomic zygote with a subsequent loss of the paternal chromosome 15 through a trisomy 15 rescue event in early pregnancy. This event allows a small number of cells to survive for embryonic development. In females, the process of X chromosome inactivation also occurs in early development and the rescue event would allow subsequent embryonic cells to have the same active X chromosome leading to X chromosome skewness. This phenomenon may lead to X linked genetic conditions in the PWS female with UPD15, if the mother is a carrier of an X linked condition.⁵

UPD15 is grouped into three separate subclasses (heterodisomy, segmental isodisomy and total isodisomy) recently identified by high-resolution SNP microarrays. Total isodisomy results from LOH of the entire chromosome 15 due to a meiosis II error or later monosomy rescue, segmental isodisomy 15 from crossover(s) in meiosis I and heterodisomy 15 when no crossover events occur.⁴⁵ The average number of crossovers between chromosome 15s occurring in meiosis I in normal individuals is approximately 1.7.¹⁸ Segmental isodisomy results when the exchange of genetic information between chromosome 15 homologues and non-disjunction occurs. The SNP microarray pattern for maternal heterodisomy 15 is the same as seen in normal (control) individuals. The presence of UPD15 and its subclass may impact diagnosis and medical care surveillance for a second genetic condition in PWS, particularly if the mother is a carrier of a recessive disorder allele in the LOH region leading to two copies of the recessive allele (eg, *POLG* gene at 15q26.1 causing mitochondrial DNA depletion). Hundreds of potentially disease-causing genes are found on chromosome 15. However, those with UPD15 due to maternal heterodisomy would not be at an increased risk. The UPD15 subclass underlines the need for an accurate, molecular genetic diagnosis for medical management and preventive care and a rationale for characterising PWS molecular classes.

High-resolution SNP microarrays can be used to identify all typical and atypical chromosome 15 deletions in PWS, and are informative in 70% of those with UPD15 (ie, segmental or total isodisomy) and also identify about one-fourth of those with imprinting centre defects. Therefore, our data when extrapolated would show that 86% of individuals with PWS have a SNP microarray abnormality. Refining the specific genetic defect and defining mechanisms for disease development and progression would be important for genetic counselling, testing at-risk individuals, in selection of prenatal testing protocols and for better treatment options, existing or novel.¹⁹

Clinical differences have been identified in those with PWS deletion subtypes but there is a paucity of data for those with imprinting defects or UPD15 subclasses. Those with segmental or total isodisomy 15 may be at greater risk for unusual clinical findings due to a second genetic disorder if the mother has a recessive or a low penetrant dominant gene allele in the region. A large LOH with more genes in the involved segment would increase the likelihood of atypical features or a second genetic condition.

There are approximately 600 protein-coding genes²⁰ on chromosome 15 with 454 annotated in OMIM (www.omim.org) including 75 autosomal recessive, 44 autosomal dominant and

125 genes for clinical disorders. With 80 Mb of DNA on chromosome 15 and an average LOH size of 25 Mb in those with maternal segmental isodisomy 15, then one would anticipate approximately 30% of the 600 genes or about 180 to be located in the segmental region. In those individuals, there is an anticipated risk of having a second genetic condition besides PWS. With this information, one should more closely examine or monitor the patient, particularly taking into consideration the chromosome segment and number of genes in the segment. Advanced genetic testing using high-resolution SNP microarrays allows for a more precise diagnosis earlier in life and leads to health surveillance for disease-causing genes on chromosome 15 impacted by the molecular class, size and location of the genetic defect in PWS.

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Figure 1.

(A) Chromosome microarray results using CNV and SNP probes to identify 15q11-q13 type I and type II deletion subtypes in Prader-Willi syndrome (PWS) with involvement of chromosome 15 breakpoints (BP1, BP2 and BP3). The circled region is not deleted in type II. (B) Methylation-specific multiplex ligation probe amplification (MS-MLPA) results of copy number comparison for PWS relative to a control subject. The circle indicates the probes (ie, *TUBGCP5*, *CYFIP1*) that are not deleted in type II. High-resolution chromosome microarrays using CNV and SNP probes to identify maternal UPD15 subclasses. (C)

Segmental isodisomy of chromosome 15. The arrows indicate areas of chromosome regions (DNA segments) with loss of heterozygosity. (D) Heterodisomy 15. Chromosome regions (DNA segments) display no loss of heterozygosity. (E) Isodisomy 15. The arrow indicates the entire length of chromosome 15 from PWS individual with loss of heterozygosity due to uniparental maternal disomy 15 (meiosis II error) or postzygotic monosomy rescue. (F) Distribution of chromosome 15 bands involved in segmental isodisomy 15 by high-resolution microarrays and dense genotyping of chromosome 15. The number of protein-coding genes in parenthesis is noted per chromosome 15 band as referenced in McGuire *et al.*²⁰

Table 1

Characterisation of Prader-Willi syndrome (PWS) genetic findings

PWs 15q11-q13 deletion n=303 (60%)		PWs maternal disomy 15 n=185 (36%)		PWS imprinting defect n=22 (4%)	
Deletion	n	Percentage	Maternal disomy 15	n	Percentage
Type I	118	38.9	Total isodisomy	13	12.5
Type II	165	54.5	Segmental isodisomy	60	57.7
Atypical	20	6.6	Heterodisomy	31	29.8
			Not established [†]	81	-

* Biparental (normal) inheritance was established using genotyping of chromosome 15 DNA markers. However, high-resolution SNP microarray analysis to determine the presence or absence of a microdeletion was not done due to lack of genetic material from the subject with PWS and/or parent(s) at the time of study. Percentage is based on the total number of 17 subjects with an imprinting defect.

[†] Maternal disomy 15 subclass was determined using high-resolution SNP microarray analysis and/or comprehensive genotyping of chromosome 15 DNA markers but not established due to lack of genetic material from the subject and/or parent(s) at the time of study. Percentage is based on the total number of 104 subjects with maternal disomy 15 subclass.