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GENETICS

Genomic imprinting disorders in humans: a mini-review

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Abstract Mammals inherit two complete sets of chromosomes, one from the father and one from the mother, and most autosomal genes are expressed from both maternal and paternal alleles. Imprinted genes show expression from only one member of the gene pair (allele) and their expression are determined by the parent during production of the gametes. Imprinted genes represent only a small subset of mammalian genes that are present but not imprinted in other vertebrates. Genomic imprints are erased in both germlines and reset accordingly; thus, reversible depending on the parent of origin and leads to differential expression in the course of development. Genomic imprinting has been studied in humans since the early 1980's and accounts for several human disorders. The first report in humans occurred in Prader-Willi syndrome due to a paternal deletion of chromosome 15 or uniparental disomy 15 (both chromosome 15s from only one parent) and similar genetic disturbances were reported later in Angelman syndrome.

Keywords Genomic imprinting · Human disorders · Assisted reproductive technology · DNA methylation · Prader-Willi syndrome · Angelman syndrome · Silver-Russell syndrome · Beckwith-Wiedemann syndrome · Albright hereditary osteodystrophy · Uniparental disomy 14

Capsule Disturbances in imprinted genes cause several human diseases involving neurological disorders, obesity, diabetes and malignancies with expression patterns of imprinted genes potentially influenced by the environment including assisted reproductive technology.

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Introduction

This mini-review includes the clinical and genetic description of five representative disorders useful from a diagnostic/clinical perspective. These include Prader-Willi and Angelman syndromes (the first examples of genomic imprinting in humans), Silver-Russell syndrome, Beckwith-Weidemann syndrome, Albright hereditary osteodystrophy and uniparental disomy 14 [1, 2]. Also, included will be an introduction and description of genomic imprinting in humans and assisted reproductive technology (ART). This review focuses on humans with limited discussion pertaining to other mammals.

Genomic imprinting is related to the methylation of cytosine bases in the CpG dinucleotides of the DNA molecule which are key regulatory elements of genes. Almost all imprinted genes have a CpG-rich differentially methylated region (DMR) which usually relates to allele repression. Many imprinted genes are arranged in clusters (imprinted domains) on different chromosomes under control of an imprinting center affecting animal growth, development and viability. Imprinted genes may also contribute to behavior and language development, alcohol dependency, schizophrenia, and possibly bipolar affective disorders. In addition, the phenomena of genomic imprinting with abnormal imprinting and loss of heterozygosity contributes to a wide range of malignancies [3–5].

The expression of imprinted genes may be tissue- and stage specific with one of the parental alleles being differentially expressed only at a certain developmental stage or in certain cells. However, the monoallelic expression of an imprinted gene is not absolute. Thus, a potential role of genomic imprinting in the differentiation of tissue types may be to determine the transcription rate of genes that influence growth through a fine balance between the expression of the two parental alleles [6].

Experimental evidence suggests that genomic imprinting evolved about 150 million years ago in a common live-born mammalian ancestor after divergence from egg-laying animals [7]. Imprinting genes provide the paternal and maternal genomes the ability to exert counteracting growth effects during embryonic development [8]. Approximately 1% of all mammalian genes are thought to be imprinted with the first gene (*H19*) reported to be imprinted in humans in 1992 [9]. Since then, many imprinted genes are now candidates for human disease including cancer, obesity and diabetes [7].

Imprinted genes are targets for environmental factors to influence expression through epigenetics whereby the expression level is altered without changing the DNA nucleotide coding structure. Imprinting disturbances have been reported in classical genetic disorders such as Beckwith-Wiedemann, Angleman and Prader-Willi syndromes while the incidence of these disorders are increased in those individuals conceived with the use of assisted reproductive technology (ART). Hence, ART may increase imprinting defects by changing the regulation of imprinted genes [10].

Epigenetics involve various processes altering gene activity without changing the primary nucleotide sequence of the DNA molecule. A common process for controlling gene activity is methylation. A gene that is methylated (inactivated) can be reactivated in male or female gametogenesis for the next generation. For example, a maternally imprinted gene (inactivated by methylation) may be unmethylated by male gametogenesis and transmitted as an active gene in the sperm.

A genome-wide search for imprinted genes in the human genome has identified over 150 candidate imprinted genes involving 115 chromosome bands [11]. The number of human diseases or disorders, due to genomic imprinting maybe greater than 100 conditions as a consequence of an inappropriate genetic alteration such as a deletion or uniparental disomy involving a gene or chromosome region. Humans are predicted to have fewer imprinted genes than mice, but the types of human genes involved are markedly different from mice [11]. Therefore, questions have been raised about the use of mice as models for human diseases, particularly those involved with imprinted genes, and assessing environmental factors that may impact on genes and their activity. Examples of classical human disorders related to alterations of genomic imprinting, besides Prader-Willi and Angleman syndromes, include Silver-Russell syndrome, Beckwith-Wiedemann syndrome, Albright hereditary osteodystrophy and, more recently, uniparental disomy 14 (both paternal and maternal forms) [5, 12–14].

Genes clustered together under the regulation of a single imprinting-controlling element suggest possible involvement of higher order regulatory elements showing allelic specific DNA replication. Genes contributed by the mother generally replicate or express at different rates than genes contributed by the father. However, inappropriate methylation may contribute to tumor formation by silencing tumor-suppressing genes or by activating growth-stimulating genes. In mammals, DNA methylation patterns are established and maintained during development by three distinct DNA cytosine methyltransferases (Dnmt1, Dnmt3a and Dnmt3b). In mammalian somatic cells, cytosine methylation occurs in 60–80% of all CpG dinucleotides that are not randomly distributed in the genome. Heavily methylated heterochromatin and repetitive sequences contribute to gene silencing. Most CpG islands located at the promoter regions of many active genes are methylation free. Understanding the functions of DNA methylation and its regulation in mammalian development will help to elucidate how epigenetic mechanisms play a role in human diseases such as neurobehavioral problems and cancer [5, 15, 16].

Many imprinted genes are growth factors such as insulin-like growth factors (e.g. *IGF2* in Beckwith-Wiedemann syndrome) or as regulators of gene expression controlling growth (e.g., the *GRB10* gene in Silver-Russell syndrome). Paternally expressed genes generally enhance growth, whereas maternally expressed genes appear to suppress growth. Imprinting disorders are associated with both genetic and epigenetic mutations or defects including disruption of DNA methylation within the imprinting controlling regions of these genes. Some patients with imprinting disorders such as Beckwith-Wiedemann syndrome may have more generalized imprinting defects with hypomethylation at several maternally methylated imprinting controlling regions disrupting growth [17, 18].

In experimental studies, manipulation of mouse embryos has resulted in diploid embryos containing only diploid paternal or maternal chromosomes. In embryos containing only a paternal genome, reduced fetal growth and a proliferative extra-embryonic (placenta) growth occurs, whereas embryos containing a diploid set of maternal chromosomes maintain a relatively normal fetal growth pattern but exhibit poor extra-embryonic growth. The process of turning on and off genes, particularly developmental genes, is ongoing throughout the life cycle in mammals influenced by tissue specificity and timing [6, 19–22].

Assisted Reproductive Technology (ART) and genomic imprinting

Although imprinted genes account for only a small proportion of the mammalian genome, they play an

important role in embryogenesis particularly in the formation of visceral structures and the nervous system [6]. Both mutations (causing DNA structure changes) and epigenetic modifications (affecting gene expression without altering the nucleotide DNA structure) in somatic cells disturb the expression of imprinted genes leading to malformations and syndromes caused by genomic imprinting defects. Therefore, manipulation of the cellular environment could interfere with regulation of expression of imprinted genes and produce an abnormal outcome. For example, in 1991, Willadsen [23] reported newborn calves produced by embryo cloning showed malformations or disturbances in growth apparently due to the inability to reprogram the somatic nucleus used in the cloning procedure. Accelerated embryo growth, increased body weight, and birth complications related to the large size were reported along with perinatal deaths [24]. Furthermore, placental abnormalities and polyhydrammos were sometimes observed in such pregnancies [25]. The large offspring size was probably due to disturbances of expression of the insulin-like growth factor receptor (Igf2r) gene [26] due to manipulations of the gametes or from the early embryos through inadequate conditions of the in vitro culturing techniques [27–29].

The use of ARTs with in vitro manipulation of gametes or from the early human embryos and potential factors impairing the expression of genes has received much attention in the medical community. According to Schieve et al. [30], infants conceived with the use of ARTs have low or very low birth weight compared to those conceived naturally. In a prospective study of Beckwith-Wiedemann syndrome (BWS), DeBaun et al. [28] reported the prevalence of ARTs as 4.6% (3 of 65 subjects) versus the background rate of 0.8% in the United States. A total of seven children with BWS were born after ART—five of whom were conceived after intracytoplasmic sperm injection. Molecular studies were performed on six of the children and five had specific imprinting or epigenetic alterations. Furthermore, in a current review of the literature on imprinting disorders and assisted reproductive technology, Manipalviratn et al. [31] found that more than 90% of children with BWS born after ART had imprinting defects compared with 40–50% of children with BWS conceived without ARTs. Independent studies in the United States, United Kingdom and France showed that the relative risk of BWS was significantly increased by a factor of 3 to 6 fold if ARTs were used in establishing the pregnancy. Patients with Angelman syndrome with complete or partial loss of methylation on chromosome 15 have also been reported to occur following the use of ARTs [32]. In addition, infants with retinoblastoma, an autosomal dominant eye tumor disorder with incomplete penetrance, have been reported following the use of ARTs [33]. Because imprinting disorders are uncommon, larger studies are needed to confirm an association between ARTs and imprinting disorders and which disorders are at the highest risk.

Examples of genomic imprinting disorders

Prader-Willi syndrome

Prader-Willi syndrome (PWS) is a complex genetic condition characterized by mental and physical findings, with obesity being the most significant health problem [34–36]. PWS is considered the most common genetically identified cause of life-threatening obesity in humans and affects an estimated 350,000–400,000 people worldwide. Prader-Willi syndrome has been estimated to occur in one in 10,000 to 20,000 individuals and present in all races and ethnic groups but reported disproportionately more often in Caucasians [34].

PWS is characterized by infantile hypotonia, early childhood obesity, short stature, small hands and feet, growth hormone deficiency, hypogenitalism/hypogonadism, mental deficiency and behavioral problems including temper tantrums and skin picking and a characteristic facial appearance with a narrow bifrontal diameter, short upturned nose, triangular mouth, almond-shaped eyes, and oral findings (sticky saliva, enamel hypoplasia) [34, 36, 37].

In 1956, Prader, Labhart, and Willi [38] were the first to report this syndrome while Ledbetter and others [39] in 1981 were the first to report an interstitial deletion of the proximal long arm of chromosome 15 in the majority of subjects. Butler and Palmer in 1983 [1] were the first to report that the origin of the chromosome 15 deletion was *de novo* or due to a new event and found that the chromosome 15 leading to the deletion was donated only from the father. In about 70% of subjects with PWS, the 15q11-q13 deletion was present while about 25% of individuals with PWS had either maternal disomy 15 (both 15s from the mother) or defects in the imprinting center controlling the activity of genes in the chromosome 15 region (about 5% of cases). Rarely, other chromosome 15q11-q13 rearrangements occur such as translocations. Occasionally, the father may have inherited an imprinting defect on chromosome 15 from his mother and can pass on the defect to his offspring at a 50% recurrence risk for PWS [36, 37].

PWS is generally divided into two major stages of clinical course development. The first stage is characterized by infantile hypotonia, temperature instability, a weak cry and poor suck, and feeding difficulties with tube feedings often required, developmental delay and underdevelopment of the sex organs. The second stage occurs in early childhood (2–4 years of age) and characterized by an insatiable appetite,

rapid weight gain and subsequent obesity without caloric restriction, continued developmental delay or psychomotor retardation. The average IQ is 65. Other features noted during the second stage include speech articulation problems, food foraging, rumination, unmotivated sleepiness, physical inactivity, decreased pain sensitivity, self-injurious behavior, strabismus, hypopigmentation, scoliosis, obstructive sleep apnea, and abnormal oral pathology [34, 40]. In addition, those with the 15q11-q13 deletion are prone to hypopigmentation and self-injurious behavior (skin picking). Those with maternal disomy 15 have higher verbal IQ scores and better memory retention (Table 1) [35].

Obesity is the most significant health problem in PWS and may be life-threatening. Weight control and dietary restrictions are key management issues with caloric intake restricted to 6 to 8 calories per centimeter of height for weight loss beginning in early childhood and to 10 to 12 calories per centimeter of height to maintain weight. The use of human recombinant growth hormone therapy has resulted in a decrease in body weight and fat, an increase in muscle mass and physical activity and a higher quality of life for PWS individuals [40].

PWS and its sister syndrome, Angelman syndrome (AS) which has an entirely different clinical presentation, were the first examples of genomic imprinting in humans. AS is characterized by seizures, severe mental retardation, ataxia and jerky arm movements, hypopigmentation, inappropriate laughter, lack of speech, microbrachycephaly, maxillary hypoplasia, a large mouth with protruding tongue, prominent nose, wide spaced teeth, and usually a maternal 15q11-q13 deletion. Although PWS is thought to be a contiguous gene syndrome with several imprinted (paternally expressed) genes as candidates for causing the disorder, AS is caused by a single imprinted (maternally expressed) gene, i.e., *UBE3A*, a ubiquitin ligase gene involved in early brain development [41]. The 15q11-q13 region contains about 6 million DNA base pairs and a large cluster of imprinted genes causing the two syndromes along with a non-imprinted domain. Novel DNA sequences have been identified with low copy repeats clustered at or near the two major proximal chromosome breakpoints (BP1 and BP2) and the distal breakpoint (BP3) in the 15q11-q13 region [42]. The typical PWS deletion consists of two classes, type I and type II, depending on the size and chromosome breakpoint position (Fig. 1). Those with the larger typical type I deletion (involving BP1 and BP3) have more clinical problems such as obsessive compulsive disorders, self-injury and poorer academic performance than those PWS subjects with the smaller type II deletions (involving BP2 and BP3) [43]. These genetic subtypes are determined by fluorescence in situ hybridization (FISH), genotyping and methylation using DNA probes from the 15q11-q13 region.

At least 70 nonredundant genes/transcripts are recognized in the 15q11-q13 region, and at least a dozen genes are imprinted and paternally expressed. Methylation DNA testing which measures the methylation status of the genes in the region can be used for laboratory diagnosis of PWS. Methylation testing is considered to be 99% accurate in the diagnosis of PWS, but does not allow for identification of the specific genetic subtype (deletion, maternal disomy or an imprinting defect). Additional testing besides FISH is required to identify maternal disomy 15 or imprinting defects such as genotyping of informative DNA markers from the 15q11-q13 region. Several genes or transcripts mapped to the 15q11-q13 region that are imprinted, with most having only paternal expression, include *SNURF-SNRPN*, small nucleolar RNAs (snoRNAs), *NDN, MKRN3* and *MAGEL2*. Candidate genes for causing PWS are paternally expressed and maternally silenced, located within the chromosome 15q11-q13 region and involved directly or indirectly in brain development and function. For example, the promoter and first exon of *SNURF-SNRPN* are integral components of the imprinting center that controls the regulation of imprinting throughout the chromosome 15q11-q13 region. A disruption of this complex locus will cause loss of function of paternally expressed genes in this region, leading to PWS [36, 37, 40, 44, 45].

Two imprinted and maternally expressed genes (*UBE3A, ATP10C*) have also been identified in this chromosome region. The *UBE3A* gene causes AS. Additional genes including the GABA receptors, *GABRB3, GABRA5,*

Table 1 Clinical and genetic findings in Prader-Willi syndrome

- First reported by Prader, Labhart and Willi [38] in 1956
- Hypotonia, poor suck, and feeding difficulties during infancy
- Characteristic face (small upturned nose, narrow bifrontal diameter, thin upper lip)
- Hyperphagia and early childhood obesity
- Hypogonadism/hypogenitalism
- Short stature, small hands and feet, growth hormone deficiency, hypopigmentation Mental deficiency (average IQ=65), behavioral problems (skin picking, obsessive-compulsive disorder)
- Genetic subtypes (e.g., maternal disomy 15, type I or type II 15q deletions) show variation in clinical phenotype
- Paternal 15q11-q13 deletion (in about 70% of cases), maternal uniparental disomy 15 (in 25%) and imprinting mutations (in 5%)

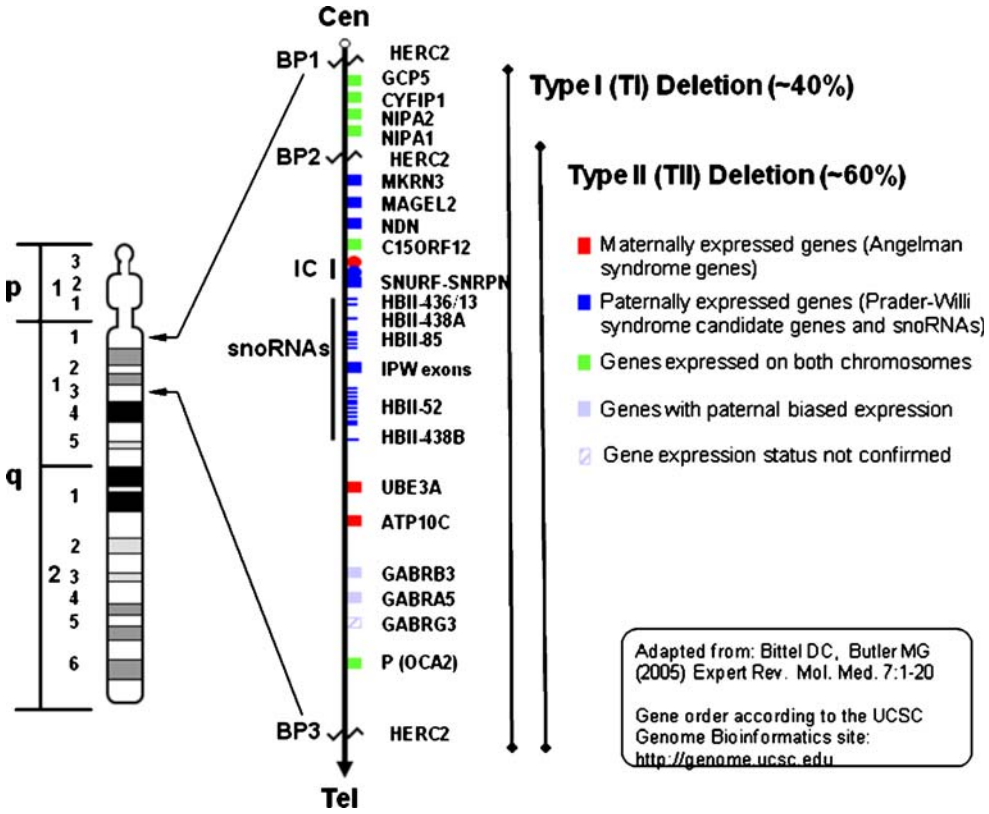

Fig. 1 Ideogram of chromosomes 15, showing genes located in the typical deletion region of Prader-Willi syndrome. The locations of genes in the region, 15q11-q13, and their imprinting status are shown. The gene disorder is based on the UCSC Genome Bioinformatics website (http://genome.ucsc.edu). Approximately 40% of subjects with the typical deletion have the larger type I deletion, and approximately 60% have the smaller type II deletion. Abbreviations: Cen, centromere; Tel, telomere; BP, breakpoint; IC, imprinting center; snoRNA, small nucleolar RNA. (Reproduced from Expert Reviews in Molecular Medicine (2005) Vol. 7, e14.)

GABRG3 and *P* (for pigmentation) have been identified in this chromosome region and not imprinted but may play a role in the PWS phenotype. Recently, a small deletion involving the paternally expressed snoRNA (HBII-85) was reported in an obese male with features of PWS, further supporting its role in the causation of PWS [46].

Maternal disomy 15 is the second most frequent finding in PWS thought due to fertilization of an oocyte with two maternal chromosome 15s by a normal sperm with one chromosome 15. This leads to a zygote which is trisomic for chromosome 15. This condition is not compatible with development and is a relatively common cause of early miscarriages. Through a trisomy rescue event in the fetus, the pregnancy is salvaged and not spontaneously aborted. This leads to a normal set of chromosomes, but with two maternal chromosome 15s in the fetus, producing PWS [47].

Silver-Russell syndrome

Silver-Russell syndrome (SRS) was first reported by Silver et al. in 1953 [48] and by Russell in 1954 [49]. SRS affects approximately 1 in 75,000 births. SRS is clinically heterogeneous with prenatal and postnatal growth retardation, a characteristic facial appearance including a small, triangular face with frontal prominence and a normal head circumference, growth asymmetry particularly of the limbs, and small incurved fifth fingers (clinodactyly). Individuals with SRS have late closure of the anterior fontanel, immature bone development and excessive sweating of the head and upper trunk during infancy. Hypoglycemia may also be present in infancy and early childhood. Patients with this disorder frequently have café au lait spots and occasionally hypospadias, cardiac defects or precocious puberty. Developmental delay can be seen. Although these patients are generally underweight and have feeding problems they gradually gain weight, but growth hormone deficiency is reported. There is a large appearing head with large fontanels in infancy resembling hydrocephalus (Table 2) [50].

Several abnormalities have been reported involving chromosomes 7, 8, 15, 17, and 18, in the form of rings, deletions, and translocations. However, the majority of Silver-Russell syndrome patients have a normal karyotype. Maternal disomy of chromosome 7, in which both chromosome 7s come from the mother, occurs in about 10% of subjects with SRS. Some SRS patients with maternal disomy 7 may have a milder phenotype [17, 50].

Although no single gene appears to be responsible for all the features seen in Silver-Russell syndrome, genetic evidence exists for involvement of two separate regions on chromosome 7 including 7p11.2-p13 and 7q31-qter. Imprinted genes with only paternal expression involving growth stimulation within the 7p13 band have been found

Table 2 Clinical and genetic findings in Silver-Russell syndrome

- First reported by Silver et al. [48] in 1953 and Russell [49] in 1954
- Small stature (prenatal onset)
- Skeletal asymmetry (in limbs)
- Characteristic face (small triangular, frontal prominence with normal head circumference, downturned corners of mouth, small chin)
- Small incurved fifth finger (clinodactyly)
- Abnormalities reported for chromosomes 7, 8, 15, 17 and 18 including rings, deletions, and translocations
- Maternal uniparental disomy 7 (in 10% of cases); 7p duplications or unknown (about 40%)
- Maternal duplication of chromosome 11p15 (5% of cases); hypomethylation of telomeric 11p15 imprinting center (40–60% of cases)

including *MEST* (mesoderm-specific transcript), *PEG1* (paternally expressed gene 1), carboxypeptidase A4 (*CPA4*), coatomer protein complex subunit gamma 2 (*COPG2*) and two imprinted noncoding RNAs (*MESTIT, C1T2/COPG2IT1*) and become potential gene candidates for this disorder.

The *GRB10* (growth factor receptor-bound protein 10) gene is maternally expressed and located in the 7p11.2-p13 region along with other genes involved in human growth and development such as *IGFBP1, IGFBP3, PHKG1, EGFR* and *GHRHR* [17, 51]. The *GRB10* gene acts as a suppressor of growth through its interaction with either the (IGF1) receptor or the growth hormone receptor [52]. In addition, two patients with SRS have been identified with cytogenetic duplications of 7p11.2-p13 encompassing the region containing the *GRB10* gene. Therefore, the explanation for maternal disomy 7 causing features of SRS specifically growth anomalies, would include two functional maternal copies (instead of one) of a growth inhibitor gene and/or the lack of paternally expressed growth promoter genes (e.g. *MEST/PEG1*). More recent studies have found genetic and epigenetic mutations affecting the imprinting centers on chromosome 11p15 in about 60% of SRS patients [53]. Therefore, SRS represents the first human disorder with imprinting disturbances affecting two different chromosomes (i.e., chromosome 7 and 11). Thus, a functional interaction of factors encoded by genes may exist between the two chromosomes. Human chromosome 11p15 contains a cluster of imprinted genes crucial for the control of fetal growth. The expression of genes in this region is regulated by two imprinting control regions (ICR1 and ICR2). The telomeric ICR1 domain controls the expression of *H19,* possibly functioning as a microRNA precursor involved in post-transcriptional regulation of specific mRNAs during vertebrate development, and *IGF2,* which is paternally expressed and involved with stimulating fetal growth and development. Chromosome 11p15 epimutations reported in SRS are typically due to hypomethylation of the ICR1 domain; this results in suppression of IGF2 growth factor activity and reduced growth in SRS patients [17, 53].

Beckwith-Wiedemann syndrome

Beckwith-Wiedemann syndrome (BWS) was first reported by Wiedemann in 1964 [54] and Beckwith in 1969 [55]. BWS is generally sporadic but an autosomal dominant transmission is reported in approximately 10–15% of cases. Major features of this syndrome are macrosomia, with a large muscle mass at birth and macroglossia, prominent eyes with periorbital fullness, and characteristic ear creases and /or pits. Other features include capillary nevus flammeus over the central forehead and eyelids; a large fontanel; accelerated bone age; growth asymmetry; organomegaly involving the kidneys, liver, pancreas, and spleen; an omphalocele; and an increased intra-abdominal tumor rate, particularly of the kidneys and occasionally the liver. Additional findings may include neonatal hypoglycemia, present in about one-third of cases, cardiovascular defects, and cryptorchidism. The mortality rate is estimated to be as high as 21%. The large tongue may interfere with breathing and cause feeding difficulties. The frequency of abdominal tumors (Wilms, hepatoblastoma) in this disorder is estimated at 10–20%. Tumor surveillance with abdominal sonograms and blood and urine biomarkers are warranted (Table 3) [50, 56].

The majority of patients with Beckwith-Wiedemann syndrome do not have a recognized chromosome abnormality but have errors in epigenetics, usually with abnormal methylation of genes in the 11p15.5 region, specifically *H19* and *IGF2.* However, the 11p15.5 chromosome band contains more than a dozen known imprinted genes, both maternal and paternal. This large domain of contiguous imprinted genes includes *IGF2* (paternally expressed), *H19* (maternally expressed), *CDKN1C* (maternally expressed), *KVLQT1* (maternally expressed), and *KCNQ10T1 (LIT1) (*paternally expressed). As noted earlier, genes in the 11p15 region are organized into two separately controlled imprinted domains; a telomeric (ICR1) and centromeric (ICR2) domain. Other target sites or binding factors in the telomeric ICR1 domain controls the transcription and regulation of *IGF2* and *H19*. Therefore, one of the most common epigenetic alterations in patients with Beckwith-Wiedemann syndrome is the abnormal (biallelic) expression of *IGF2* or

Table 3 Clinical and genetic findings in Beckwith-Wiedemann syndrome

- First reported by Wiedemann [54] in 1964 and Beckwith [55] in 1969
- Macrosomia with large muscle mass at birth
- Craniofacial features (macroglossia, prominent eyes, periorbital fullness, ear creases and/or pits)
- Omphalocele, hypoglycemia
- Organomegaly (kidneys, liver, spleen), abdominal tumors
- Hemihypertrophy
- Paternal uniparental disomy 11 (in 15% of cases); loss of imprinting of *IGF2* (hypermethylation of telomeric imprinting center region) (in 5%); mutations in *CKN1C* in centromeric imprinting center region (in 10%); hypomethylation of centromeric imprinting center region (about 50%); unknown (15%)

insulin-like growth factor 2 gene encoding a fetal mitogen which stimulates growth. This abnormal expression is due to loss of imprinting. Thus, there appears to be a reciprocal coordinated relationship between the insulin-like growth factor 2 (*IGF2)* and *H19* genes in cellular growth and development. The maternally expressed *H19* gene encodes a polyadenylated-spliced message and is assumed to act as a growth-suppressing agent [17, 18, 57].

Mechanisms that increase expression of *IGF2* include maternally derived translocations and inversions of chromosome 11p15, duplications of the paternal chromosome 11p15, paternal disomy 11 (10–20% of cases of BWS) and imprinting anomalies; all lead to BWS. Hypermethylation of the ICR1 domain accounts for about 5% of BWS cases. The centromerically located ICR2 domain regulates the expression of *CDKN1C, KCNQ1* and other genes on the maternal allele. The gene of another non-coding RNA in 11p15, *KCNQ1OT1 (LIT1),* is localized in intron 9 of the *KCNQ1* gene and expressed on the paternal allele. It probably represses the CDKN1C gene. Loss of methylation of the maternal ICR1 domain correlates with expression of *KCNQ1OT1 (LIT1)*. Mutations of the *CDKN1C* gene account for about 40% of familial BWS cases and 5–10% of sporadic cases. In BWS, ICR2 hypomethylation and *CDKN1C* point mutations lead to reduced expression of *CDKN1C* and overgrowth. Finally, loss of imprint of *KCNQ1OT1 (LIT1)* accounts for about 50% of BWS cases [18].

Phenotype/genotype studies have shown an association of hemihypertrophy and hypoglycemia in BWS, with altered methylation of both the *KCNQ1OT1 (LIT1)* and *H19* genes. Patients with Beckwith-Wiedemann syndrome and tumors have been described with an altered *H19* gene methylation. In addition, an association has been reported with macrosomia and midline abdominal wall defects and altered methylation of the *KCNQ1OT1 (LIT1)* transcript. Therefore, the imprinting interaction of contiguous genes clustered in the 11p15.5 region involved in this overgrowth syndrome and the genetically opposite effects seen in Silver-Russell syndrome will require additional studies for clarification and understanding.

Albright hereditary osteodystrophy

[Pseudohypoparathyroidism (PHP),
Pseudopseudohypoparathyroidism (PPHP)]

Albright [58] first reported this osteodystrophy condition in 1942 which is due to an end-organ resistance to the actions of parathyroid hormone (PTH) and other hormones. Two major variants have been described: PHP (PHP-Ia, PHP-Ib) and PHPP. Individuals with PHP-Ia have features of Albright hereditary osteodystrophy (AHO) and present with hypocalcemia and hyperphosphatemia despite elevated serum parathyroid hormone levels. Resistance to thyroid stimulating hormone and gonadotropins as well as growth hormone-releasing hormone and calcitonin can also occur in these affected individuals. Individuals with PPHP have the characteristic physical features of AHO, but show no evidence of resistance to parathyroid hormone or other hormones. PHP-Ia and PPHP have been reported in the same families, but are dependent on the parent of origin. Both variants result from decreased activity of the alpha subunit of the membrane bound trimeric G subunit-regulatory protein (GNAS). The function of this guanine nucleotide-binding signaling protein is to couple membrane receptors for adenyl cyclase activity thereby stimulating the secondary messenger, cyclic adenosine monophosphate (cAMP) [50, 59].

Genetic defects are associated with different forms of this condition by involving the *GNAS* gene located at chromosome 20q13.11. *GNAS* is a complex imprinted gene that produces multiple transcripts through the use of alternative promoters and alternative splicing. It encodes four main transcripts: G protein subunit alpha (involved in AHO), XLAS (paternally expressed), NESP55 (maternally expressed and encodes a chromogranin-like neuroendocrine secretory protein) and the A/B transcript (derived from the paternal *GNAS* allele). *GNAS* is involved in the pathophysiology of these disorders through complex mechanisms and pathways [60].

The clinical features of AHO consist of small stature (final adult height 54 to 60 inches), moderate obesity, mental deficiency (average IQ of 60), round face with a short nose and short neck, delayed dental eruption and

Table 4 Clinical and genetic findings in Albright Hereditary Osteodystrophy (AHO) [Pseudohypoparathyroidism (PHP); Pseudopseudohypoparathyroidism (PPHP)]

- First reported in 1962 by Albright et al. [58]
- Small stature (final height, 54 to 60 inches) and short metacarpals
- Rounded face with short neck
- Delayed dental eruption or enamel hypoplasia
- Areas of mineralization in subcutaneous tissues with variable hypocalcemia and hyperphosphatemia
- Defects of the *GNAS* gene associated with different forms of PHP and PPHP depending on the parent of origin. For example, maternal inheritance leads to PHP-Ia, i.e., AHO plus hormone resistance while paternal inheritance leads to PHPP or AHO without evidence of resistance to parathyroid hormone

enamel hypoplasia, short metacarpals and metatarsals especially of fourth and fifth digits, short distal phalanx of the thumb, osteoporosis, areas of mineralization in subcutaneous tissues including the basal ganglia, variable hypocalcaemia and/or hyperphosphatemia and seizures. Occasional findings include hypothyroidism, hypogonadism, lens opacity or cataracts, optic atrophy, ocular degeneration and vertebral anomalies (Table 4) [50, 61, 62].

Patients with PHP are subdivided into PHP-Ia and PHP-Ib, depending on the presence or absence of additional hormone resistance and the AHO phenotype. Nearly all patients with PHP-Ia have mild hypothyroidism, hypogonadism and abnormal response to growth hormone releasing hormone while those patients with PHP who present with PTH-resistance, but lack AHO features are defined as having the PHP-Ib subtype. Most PHP-Ib cases are sporadic, but some have occurred in families with an autosomal dominant inheritance pattern with incomplete penetrance. Patients with PHP-Ib typically lack *GNAS* gene mutations; however, studies show that the inheritance comes from a female exhibiting alteration in imprinting of the *GNAS* locus. The most consistent defect is loss of methylation in controlling elements regulating the imprint of the *GNAS* gene. In addition, a case of PHP-Ib was found with paternal disomy of chromosome 20 [59].

Those patients with PHP-Ia and features of AHO are reported with mutations of the *GNAS* gene as well as cytogenetic deletions of chromosome 20q including *GNAS*. Patients with PHPP (or those AHO patients without evidence of hormone resistance) also carry heterozygous inactivating *GNAS* mutations. Interestingly, maternal inheritance of such a mutation can lead to PHP-Ia (AHO with hormone resistance) while paternal inheritance of the same mutation leads to PHPP or AHO alone. The nature of the imprinted mode of inheritance for hormone resistance could be explained by the predominantly maternal expression of *GNAS* in certain tissues. Patients with PHP-Ia lacking *GNAS* mutations, but display the gene disturbance, are due to an imprinting defect and loss of imprint at the exon A/B differentially methylated region (DMP) of the gene. In addition, a unique 3-Kb microdeletion that disrupts the neighboring STX 16 close to the differentially methylated domain can cause PHP-I as well and loss of imprint [59, 60].

In summary, the pattern of inheritance of the *GNAS* gene located at chromosome 20q13.11 that stimulates adenyl cyclase activity is responsible for both PHP-Ia and PPHP variants of the AHO syndrome with multiple transcriptional units. PHP-Ia and PPHP are caused by heterozygous inactivating mutations in those exons of the *GNAS* gene encoding the alpha subunit of the stimulatory guanine nucleotide-binding protein and the autosomal dominant form of PHP-Ib is caused by heterozygous mutations disrupting a long-range imprinting control element of *GNAS*. Both disorder variants have been reported in the same family and dependent on parent of origin, therefore due to imprinting. If the altered gene is inherited from the affected father with either PHP-Ia or PPHP, then PHPP occurs in the offspring. If the inheritance of the same *GNAS* mutation is present in the mother with either PHP-Ia or PHPP, then the child will present with PHP-Ia.

Table 5 Clinical and genetic findings in uniparental disomy 14 (maternal and paternal)

- First reported in 1991 by Wang et al. [63] and Temple et al. [64]
- Clinical findings in maternal disomy 14 include growth retardation, congenital hypotonia, joint laxity, psychomotor retardation, truncal obesity and minor dysmorphic facial features
- Clinical features are more severe in paternal disomy 14 including polyhydramnios, thoracic and abdominal wall defects, growth retardation and severe developmental delay.
- Imprinting errors with imprinted locus at 14q32 including the paternally expressed *DLK1* gene and maternally expressed *GTL2* gene
- Uniparental disomy, copy number changes and disruption of regulatory sequences or mutations of a single active allele leads to the disorder

Uniparental disomy 14

Wang et al. [63] and Temple et al. [64] in 1991 described different clinical phenotypes in those subjects with either paternal or maternal disomy of chromosome 14. Maternal disomy 14, the inheritance of both chromosome 14 homologues from the mother often involves a chromosome 14 translocation, but may have features in common with Prader-Willi syndrome [13, 65]. Maternal disomy 14 is characterized by prenatal and postnatal growth retardation, congenital hypotonia, joint laxity, gross motor delay with mild to moderate mental retardation, early onset of puberty, truncal obesity and minor dysmorphic features of the face, hands and feet. About 30% of cases will show rapid postnatal head growth usually due to hydrocephalus that is arrested spontaneously. Dysmorphic facial features include a prominent forehead, prominent supra-orbital ridges, a short philtrum and down-turned corners of the mouth [13]. Over 30 cases have been reported. Paternal disomy 14 has a more severe presentation including polyhydramnios, thoracic and abdominal wall defects, growth retardation and severe developmental delay. Errors in imprinting of chromosome 14 are likely causes of the phenotypes while segmental uniparental disomy 14 has been reported involving the distal chromosome 14q region indicating a critical area for the phenotype (Table 5) [13, 14, 66].

An imprinted locus existing at 14q32 appears to be under the control of a paternally methylated region. Imprinted genes in this region include the paternally expressed *DLK1* (delta, Drosophila homologue-like 1), a transmembrane signaling protein which is a growth regulator homologous to proteins in the Notch/delta pathway [14]. A maternally expressed gene *GTL2,* gene trap locus 2, and a large non-coding RNA cluster are also present in the region. Therefore, the clinical phenotypes of maternal and paternal disomy of chromosome 14 appears to be due to dysregulation of imprinted genes from several mechanisms including uniparental disomy, copy-number change in the imprinted genes, disruption of regulatory sequences or mutations of a single active allele. Chromosome and molecular studies are needed including methylation testing, genotyping and chromosome microarray hybridization in those individuals presenting with congenital hypotonia, unexplained growth and psychomotor retardation and dysmorphic features in order to rule out uniparental disomy 14 or other uniparental disomic syndromes such as Prader-Willi syndrome.

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