REVIEW

Role of DNA methylation in imprinting disorders: an updated review

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Abstract Genomic imprinting is a complex epigenetic process that contributes substantially to embryogenesis, reproduction, and gametogenesis. Only small fraction of genes within the whole genome undergoes imprinting. Imprinted genes are expressed in a monoallelic parent-of-origin-specific manner, which means that only one of the two inherited alleles is expressed either from the paternal or maternal side. Imprinted genes are typically arranged in clusters controlled by differentially methylated regions or imprinting control regions. Any defect or relaxation in imprinting process can cause loss of imprinting in the key imprinted loci. Loss of imprinting in most cases has a harmful effect on fetal development and can result in neurological, developmental, and metabolic disorders. Since DNA methylation and histone modifications play a key role in the process of imprinting. This review focuses on the role of DNA methylation in imprinting process and describes DNA methylation aberrations in different imprinting disorders.

Keywords DNA methylation · Genomic imprinting · Prader–Willi syndrome · Silver–Russell syndrome · Beckwith–Wiedemann syndrome · Type 1b pseudohypoparathyroidism

Abbreviations

DMRs differentially methylated regions
ICR imprinting control region
IGF2 insulin-like growth factor 2

UPD	uniparental disomy
ER	epigenetic reprogramming
ART	assisted reproductive technology
MLID	multi-locus imprinting disturbances
TNDM	transient neonatal diabetes mellitus
PWS	Prader–Willi syndrome
SRS	Silver–Russell syndrome
PHP1b	pseudohypoparathyroidism type 1b
AS	Angelman syndrome

Introduction

Genomic imprinting (GI) is a unique phenomenon that occurs in placental mammals, marsupials, and a subset of flowering plants. Genomic imprinting plays a key role in maintaining normal embryogenesis, and prenatal and postnatal growth. In genomic imprinting, several epigenetic processes are involved to result in a unique epigenetic signature observed at a subset of loci in the genome [1, 2]. DNA methylation is the mainstay of establishing imprinting marks on either paternal or maternal alleles. Besides, histone modifications and non-coding RNAs contribute greatly to imprinting process [3, 4]. The predominant feature of genomic imprinting is the monoallelic gene expression in parent-of-origin-specific manner. It means that only one of the two inherited alleles is expressed either from the paternal or maternal side. When paternal allele is expressed, maternal copy is silenced, and vice versa [5].

Genomic imprinting is a complex form of epigenetic inheritance. One feature of the complexity of genomic imprinting is that it works in tissue-specific manner. For example, *UBE3A* is only imprinted in human brain where it is maternally expressed, while biallelic expression of *UBE3A* was reported in many tissues and cell lines apart from the brain [6, 7]. Another example is the tissue-specific imprinting of *Gs*–

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alpha from *GNAS* locus on chromosome 20q13. Gs-alpha is only maternally expressed in the renal tubules while it is expressed biallelically from most tissues [8]. Time-based or temporal dependence is an additional significant feature of imprinting, i.e., certain genes are biallelically expressed during embryogenesis while these genes are imprinted in parent-of-origin-specific manner after adulthood. For example, *IGF2* is biallelically expressed in the fetal brain during the first trimester while *IGF2* is expressed predominantly from the paternal allele in other tissues. However, during adulthood, certain parts of the human brain, such as the hypothalamus and globus pallidus, show paternal monoallelic expression of *IGF2*, whereas the pons shows biallelic expression of *IGF2* [9]. Furthermore, individual genes often produce several transcripts, which can show different expression patterns; for example, some transcripts are imprinted and other transcripts are non-imprinted. *PLAGL1* locus on chromosome 6q24 gives a good demonstration of this complex behavior of imprinting. *PLAGL1* is composed of 12 exons with four different promoters. *PLAGL1* transcripts initiated from promoters 2, 3, and 4 are biallelically expressed in most tissues, whereas only *PLAGL1* transcripts initiated from promoter 1 (ICR/P1) are paternally expressed [10].

Interestingly, several imprinted genes are conserved among different species (human, mice, pigs, etc.). According to the most recent studies, about 127 imprinted genes in mice and around 94 imprinted genes in human have been reported and identified [11, 12]. Most of these imprinted genes are grouped together in clusters and loci. Each locus has at least one differentially methylated region (DMR). DMRs in most imprinted loci comprise CpG-rich regions called imprinting control regions (ICRs), which work as cis-acting regulatory elements controlling imprinted genes. ICRs are characterized by their monoallelic DNA methylation and distinct histone marks that achieve monoallelic parent-of origin-specific expression [13, 14].

Epigenetic reprogramming and imprinting

After fertilization, male and female pronuclei go through several changes before their fusion. One of these eminent changes is epigenetic reprogramming (ER) which plays a crucial role in early embryogenesis and gametogenesis. ER is a dynamic process of erasing and reestablishing epigenetic marks in embryonic genome during the early embryonic development. ER occurs in two cycles or waves. The first cycle occurs during the blastocyst stage and the second cycle occurs in the primordial germ cell development. There are specific regions of the genome that undergo different conditions of ER, e.g., imprinted genes and most repetitive elements [15, 16].

Most repetitive elements maintain their DNA methylation patterns throughout the embryonic development without change. Similarly, imprinted genes evade the first cycle of epigenetic reprogramming by maintaining their epigenetic marks. However, imprinted genes undergo resetting of these epigenetic marks during the stage of primordial germ cell development. Reestablishing these marks occurs in parent-of-origin-specific manner [17, 18]. Therefore, any error or defect in the process of epigenetic reprogramming of ICRs can result in loss of imprinting [19, 20].

DNA methylation and genomic imprinting

Imprinting is of epigenetic nature rather than DNA sequence dependent. Imprinting marks are established in gametes during primordial germ cell development in a parent-of-origin-specific manner. In mammals, DNA methylation and histone modifications play crucial role in establishing the imprinting marks [14, 21]. In imprinted loci, ICRs control expression of imprinted genes in unique mechanisms depending on ICR position. ICRs are found either at the intergenic region (known as intergenic ICRs) or at the promoter region of certain gene within the imprinted locus (known as promoter ICRs) [22, 23]. Many promoter ICRs control the expression of non-coding RNA that regulates imprinting of other genes in the imprinted locus. For example, ICR2 is located in the promoter region of *KCNQ1OT1* and regulates the *CDKN1C/KCNQ1* locus. *KCNQ1OT1* is a long non-coding RNA, which silences genes located in cis and functions in a similar manner to Xist RNA [24–26]. Typically, ICR2 is maternally methylated and paternally unmethylated. Therefore, *KCNQ1OT1* RNA expression is maternally silenced, whereas *KCNQ1OT1* is paternally expressed. As a result, imprinted genes of the *CDKN1C/KCNQ1* locus are maternally expressed and paternally silenced [27].

Intergenic ICRs generally regulate imprinting in a different way through functioning as a transcription insulator in the imprinted cluster. For example, ICR1, which controls *H19/IGF2* cluster, exists in the intergenic region between *H19* and *IGF2* genes. DNA hypomethylation of ICR1 assists the binding of the transcriptional repressor protein called CCCTC-binding factor (CTCF) that insulates *IGF2* from downstream enhancers. Without CTCF binding, the downstream enhancers can access *IGF2* promoter resulting in expression of *IGF2* [28, 29]. Typically, paternal ICR1 of the *H19/IGF2* cluster is hypermethylated, whereas maternal ICR1 is hypomethylated. Therefore, insulin-like growth factor 2 (*IGF2*) is monoallelically expressed from the paternal allele and silenced in the maternal allele. Interestingly, most promoter ICRs are maternally methylated while several intergenic ICRs are methylated on the paternal side [30, 31].

Imprinting disorders

Imprinting disorders always result from loss of imprinting in the key imprinted loci, whereas loss of imprinting does not always result in imprinted disorders. For example, loss of imprinting of H19 and IGF2 has been reported in phenotypically healthy infants, and this reflects a broad spectrum of phenotypes that can be observed in association with loss of imprinting [32]. Loss of imprinting results from specific four causes, which are deletions, mutations, uniparental disomy, and epimutations. These causes affect the imprinted loci and result in aberrant silencing of the active allele or expression of the inactive allele. Mutations or deletions are common causes of imprinting disorders and can affect genes, promoters, intergenic regions, and ICRs within the imprinted loci [33, 34]. Uniparental disomy (UPD) represents another cause of imprinting disorders. UPD refers to the abnormal receiving of a chromosome pair from one parent and no chromosome for this pair from the other parent. UPD results in inheriting two paternal copies of chromosome or two maternal copies [35]. Epigenetic disruption or epimutation is also a significant cause for loss of imprinting. Epimutations can result from aberrations of DNA methylation in ICRs or disruptions in histone modifications within the imprinted clusters [36, 37].

Since environmental factors can affect epigenetic marks throughout the genome altering gene expression. Similarly, using assisted reproductive technology (ART) can affect epigenetic marks in the imprinted loci disrupting genomic imprinting and altering expression of imprinted genes. ART has been associated with an increased incidence of imprinted disorders, e.g., Angleman, Prader–Willi, and Beckwith–Wiedemann syndromes, compared to natural conception [38–40]. However, the absolute risk of developing imprinting disorders in ART-conceived children was low, and the combined odds ratio of any imprinting disorder in ART-conceived children was 3.67 (95% confidence interval, 1.39–9.74). Moreover, there was no significant association between ART and the methylation status of imprinted genes in the same meta-analysis [41]. Therefore, further investigations are warranted to unravel the correlation of ART with epigenetic changes and dysregulation of genomic imprinting, and to find out the absolute risk of developing imprinting disorders using different protocols of ART.

Imprinting disorders were firstly identified as separate disorders with their unique phenotypes and (epi)genetic causes (Table 1). However, recent studies reported that some patients with specific ID show multi-locus imprinting disturbances (MLID) in other imprinted loci [42, 43]. MLID were first reported in transient neonatal diabetes mellitus (TNDM) patients, who showed mosaic DNA hypomethylation in other imprinting regions other than the *PLAGL1* locus on chromosome 6q24.2 [44]. About 50% of TNDM patients, who had hypomethylation of ICR/P1, reported another epimutation on

chromosome 11p15 (i.e., hypomethylation of ICR2 or KvDMR1 which is very common epimutation in Beckwith–Wiedemann syndrome (BWS) patients) [45]. Similarly, MLID have been reported in other imprinting disorders but with lower frequency than TNDM, for example, Silver–Russell syndrome (SRS), pseudohypoparathyroidism type 1b (PHP1b), Angelman syndrome (AS), and Beckwith–Wiedemann syndrome [46–49]. Intriguingly, MLID can occur on either maternal or paternal imprinted regions with either hypomethylation or hypermethylation of DMRs. In a special case, TNDM patients, who showed MLID, reported only hypomethylation in other imprinting loci with no reports on hypermethylation [42, 50]. This review discusses different imprinting disorders and the underlying (epi)genetic causes in detail.

Transient neonatal diabetes mellitus

Transient neonatal diabetes mellitus (TNDM) is a growth retardation syndrome that is associated with persistent hyperglycemia during the first 6 weeks of life because of insulin deficiency. Recovery occurs in approximately 50% of these neonates after 18 months of birth. The only treatment of TNDM is exogenous insulin during persistent hyperglycemia. TNDM has a very low incidence (about 1 in 400,000 births in UK) [83–85]. Besides, neonates, who had TNDM, are more likely to develop type 2 diabetes (T2DM) later in life. TNDM has three types according to the responsible genes. TNDM1 is associated with defect in the expression of pleomorphic adenoma gene-like 1 (*PLAGL1*) on 6q24.2 locus and to lesser extent, the expression of zinc finger protein 57 homolog (*ZFP57*) on 6p22.1 locus. Meanwhile, TNDM2 and TNDM3 are associated with mutations in *ABCC8* on chromosome 11p15.1 and *KCNJ11* on chromosome 11p15.1 locus, respectively. TNDM1 is an imprinted form of TNDM, whereas TNDM2 and TNDM3 are non-imprinted forms [86–89]. Paternal UPD of chromosome 6 (35–40% of TNDM1) and duplication of 6q24 (35–40% of TNDM1) are the main mechanisms of TNDM1 [85, 90, 91]. However, aberrant DNA methylation in the ICR/P1 of 6q24 locus occurs in about 20% of TNDM1 patients.

ZFP57 encodes a transcription repressor which recognizes specific DNA sequences and forms a complex with co-repressor protein called KRAB-associated protein-1 (KAP1) [92, 93]. Kap1 recruits other proteins, for example, histone H3-K9 methyltransferase-4 (SETDB1) and nuclear protein 95 (NP95), which in turn recruit DNA methyltransferases (DNMTs). Hence, this complex plays a crucial role in regulating and maintaining DNA methylation at different ICRs [94, 95]. Either mutation or 1-bp deletion in *ZFP57* gene can give rise to a truncated protein, which losses its function as a regulator of DNA methylation at ICRs. As a result, MLID and hypomethylation occur in certain imprinted loci leading to

Table 1 Overview of different imprinting disorders in humans

Imprinting disorder	Imprinted domain	Cytogenetic abnormality	Uniparental disomy	Imprinting center epimutation	Deletions/duplications involving imprinting center	Gene mutation	MLID	References
Transient neonatal diabetes 1 (TNDM1, OMIM 601410)	6q24	Visible paternal duplication of 6q24 (2%)	Paternal UPD6 (35–40%)	Hypomethylation of the maternal ICR/P1 (20%)	Submicroscopic duplication of the 6q24 region on the paternal allele (35–40%)		Reported	[45, 51–53]
Silver–Russell syndrome (SRS, OMIM 180860)	11p15.5	Maternal duplication of 11p15 (<2%)	Maternal UPD11 (1 case)	Hypomethylation of the paternal IC1 (up to 70%)	IC2 duplication on maternal allele (1 case)		Reported	[54–59]
Beckwith–Wiedemann syndrome (BWS, OMIM 130650)	7p12.2, 7q32.2 11p15.5	Chromosomal rearrangement (<2%) Chromosomal rearrangement (1–2%)	Maternal UPD7 (5%) Paternal UPD11 (20%)	• Hypermethylation of the maternal IC1 (5%) • Hypomethylation of the maternal IC2 (50%)	• IC1 deletion on maternal allele (<5%) • IC2 deletion on maternal allele (<2%)	CDKN1C on maternal allele (5% sporadic, 50% in familial cases)	Reported	[29, 46, 60–65]
Prader–Willi Syndrome (PWS, OMIM 176270)	15q11–q13	• Deletions of 15q11.2–q13 on maternal allele (65–75%) • Chromosomal rearrangement (<1%)	Maternal UPD15 (25–30%)	• 1% of PWS is due to imprinting defects (epimutations) • Hypermethylation of paternal PWS/ICR (75–80% of imprinting defects cases)	Deletions in the regulatory region located around PWS/ICR on the paternal allele (15% of imprinting defect cases)		Not reported	[66–70]
Angelman syndrome (AS, OMIM 105830)	15q11–q13	Deletions of 15q11.2–q13 on maternal allele (70%) Chromosomal rearrangement (<1%)	Paternal UPD15 (7%)	• 2–5% of AS is due to imprinting defects (epimutations) • Hypomethylation of the SNRPN DMR on the maternal allele (80–90% of imprinting defects cases)	Deletions in the regulatory region located around AS/ICR on the maternal allele (10–20% of imprinting defect cases)	UBE3A on maternal allele (11%)	Reported once	[66, 67, 71–75]
Pseudohypoparathyroidism type 1b (PHP1B, OMIM 603233)	20q13	Microdeletion in STX16 (in about 20% of PHP1B patients with exon 1A ICR hypomethylation)	Paternal UPD20 (few cases reported)	Hypomethylation of ICR of exon 1A on the maternal allele (sporadic cases)		Deletion in NESPAS and NESP55 (very few familial cases)	Reported	[47, 48, 76–82]

loss of imprinting. However, *ZFP57* mutations are rare and were reported in only few cases of TNDM1 [43, 47, 50, 53].

PLAGL1 is a transcription regulator and tumor suppressor candidate gene encoding a zinc finger protein, which controls cell cycle and apoptosis. Zinc finger protein *PLAGL1* has another role in inducing the transcription of pituitary adenylate cyclase-activating polypeptide (PACAP) receptor, which enhances insulin secretion and works as a regulator of pancreatic β -cells [96, 97]. Therefore, overexpression of *PLAGL1* results in upregulation of PACAP receptor expression, which in turn deregulates the function of pancreatic β -cells and causes the development of TNDM1 [98–100]. Recent reports, which studied intrauterine growth restriction (IUGR) in human and mice, have revealed the novel role of *PLAGL1* as a main regulator to various imprinted and non-imprinted genes network involved in cellular growth and metabolism. Therefore, up- or downregulation of *PLAGL1* has been associated with altered expression levels of certain genes regulating metabolism, e.g., *IGF2*, *H19*, *SLC2A4*, *CDKN1C*, and *PPAR γ 1* [101, 102]. This suggests an alternative mechanism of developing TNDM1 due to overexpression of *PLAGL1*.

PLAGL1 comprises of 12 exons controlled by four distinct promoters producing different transcripts and isoforms. The biallelic transcripts of *PLAGL1* arise from promoters P2, P3, and P4, while only the *PLAGL1* transcripts from the ICR/P1 promoter are paternally expressed [10]. Another important gene on *PLAGL1* locus is *HYMAI*, which encodes a long non-coding RNA (lncRNA). *HYMAI* is one-exon gene that has an overlapping transcription start site with the *PLAGL1* transcripts from the ICR/P1 promoter. Therefore, the methylation status of ICR/P1 promoter regulates the expression of *HYMAI* and certain *PLAGL1* transcripts (Fig. 1) [103, 104]. Typically, the paternal ICR/P1 is hypomethylated while the maternal ICR/P1 is hypermethylated. Therefore, *HYMAI* and *PLAGL1* transcripts, which are regulated by ICR/P1 promoter, are paternally expressed and maternally silenced [105]. Several studies have reported the loss of methylation in the maternal allele in TNDM1 patients and its correlation with higher weight and body mass index [106, 107]. Aberrant hypomethylation of ICR/P1 on the maternal allele results in overexpression of *PLAGL1* and *HYMAI*, which accounts for about 20% of TNDM1 patients [51, 53]. Moreover, recent studies have shown the association between the methylation status of *PLAGL1* promoter on chromosome 6 and different types of cancer, e.g., ovarian cancer and soft-tissue sarcomas [108, 109].

Silver–Russell syndrome

Silver–Russell syndrome (SRS) is a very rare growth retardation disorder characterized by dwarfism, triangular face, congenital hemihypertrophy (asymmetric body), and low birth weight. Malnutrition and hypoglycemia commonly occur in

SRS patients. Incidence rate of SRS is approximately 1 in 100,000 births in the USA [110]. SRS is a distinct genomic imprinting disorder because the pathogenesis of SRS depends primarily on epimutation and methylation status of ICR1 at *H19/IGF2* cluster. In addition to epimutation, maternal uniparental disomy of chromosome 7 (UPD7) has been reported in some patients showing less severe phenotype of SRS [111–113]. Intriguingly, ICR1 hypomethylation of *H19/IGF2* cluster on chromosome 11p15 occurs in SRS while hypermethylation of ICR1 is found in roughly 10% of Beckwith–Wiedemann syndrome (BWS) patients [30, 114].

Normally, paternal ICR1 in *H19/IGF2* cluster is hypermethylated while maternal allele is hypomethylated. Hypomethylation of the maternal allele triggers *H19* expression and silences expression of maternal *IGF2*. Hypermethylation of ICR1 impedes its binding to the transcriptional repressor CTCF leading to *IGF2* expression and silencing of *H19* expression [115, 116]. Therefore, *IGF2* is entirely expressed from the paternal allele while *H19* is expressed predominantly from the maternal side. Insulin-like growth factor 2 (IGF2) is an important hormone contributing in cellular proliferation and growth promotion. In SRS, hypomethylation of paternal ICR1 prevents *IGF2* expression triggering defective prenatal development and growth retardation. However, there is no correlation between the level of ICR1 hypomethylation and the severity of SRS symptoms [30, 113].

Beckwith–Wiedemann syndrome

Beckwith–Wiedemann syndrome (BWS) is a rare imprinting disorder classically characterized by prenatal and postnatal overgrowth. BWS patients have distinctive manifestations, e.g., macroglossia (large tongue), macrosomia (higher birth weight than average), abdominal wall defects, hemihypertrophy (asymmetric body), and neonatal hypoglycemia. BWS has low incidence rate worldwide, i.e., about one in every 13,700 birth [61, 117]. Approximately 10% of BWS patients could develop pediatric malignancies, e.g., hepatoblastoma and Wilms tumor [118, 119]. These malignancies occur mostly before age of four; therefore, routine abdominal ultrasound and serum alpha-fetoprotein (AFP) measurements are warranted from birth until this age [120]. In BWS, epimutation of ICR1 or ICR2 accounts for about 50–60% of all cases, while paternal UPD11 accounts for 20% of cases. Additionally, *CDKN1C* mutation is responsible for 5% of sporadic cases and about 50% of familial BWS patients [29, 65].

CDKN1C/KCNQ1 cluster and *H19/IGF2* cluster within chromosome 11p15 are the key players in most BWS patients. *CDKN1C/KCNQ1* cluster is an imprinted genes cluster located centromerically to the *H19/IGF2* cluster and controlled by ICR2 or KvDMR1. DNA methylation status of ICR2 decides whether paternal or maternal copy of genes will be expressed [121]. As we discussed before, *CDKN1C/KCNQ1* cluster is regulated by the expression of a long non-coding RNA called

Fig. 1 Imprinting at the *PLAGL1* locus. **a** *PLAGL1* is composed of 12 exons with four different promoters; *PLAGL1* transcripts from promoter 1 (ICR/P1) are only expressed paternally. In contrast, *PLAGL1* transcripts from P2, P3, and P4 are biallelically expressed in most tissues. *HYMAI* is one-exon gene encoding a long non-coding RNA (lncRNA). *HYMAI* exon is also expressed paternally due to sharing promoter 1 (ICR/P1) with *PLAGL1*. Typically, *HYMAI* and ICR/P1 transcripts of *PLAGL1*

are silenced on the maternal allele due to the methylated ICR/P1. On the other hand, the paternal ICR/P1 is unmethylated allowing *HYMAI* and ICR/P1 transcripts of *PLAGL1* to be expressed. **b** In transient neonatal diabetes mellitus (TNDM1), ICR/P1 transcripts of *PLAGL1* and *HYMAI* are biallelically expressed due to loss of methylation in the maternal allele or duplication of 6q24 on the paternal allele or paternal UPD6

KCNQ1-overlapping transcript 1 (KCNQ1OT1) or long QT intronic transcript 1 (LIT1). *KCNQ1OT1* functions as an antisense to *KCNQ1* and other genes in the *CDKN1C/KCNQ1* domain silencing their expression. When ICR2 on an allele is hypomethylated, *KCNQ1OT1* is expressed and silences this allele (Fig. 2) [122, 123]. Besides, cyclin-dependent kinase inhibitor 1C (*CDKN1C*) functions as a tumor suppressor gene inhibiting G1 cyclin/CDK complexes [124, 125].

Normally, paternal ICR2 is hypomethylated while maternal ICR2 is methylated. Therefore, paternal *KCNQ1OT1* is expressed and is blocking expression of *CDKN1C/KCNQ1* domain. Whereas, methylation of maternal ICR2 prevents *KCNQ1OT1* expression allowing *CDKN1C* to be maternally expressed. In about 50% of BWS patients, maternal ICR2 is aberrantly hypomethylated besides the normally hypomethylated paternal ICR2 [126]. Consequently, *KCNQ1OT1* is expressed biallelically resulting in diminished expression of *CDKN1C* [127].

In addition to epimutation of *CDKN1C/KCNQ1* domain, aberrant DNA methylation of ICR1 in *H19/IGF2* cluster occurs in roughly 5–10% of BWS patients [61, 128]. Normally, *IGF2* is paternally expressed due to hypermethylation of ICR1. Aberrantly, hypermethylation of maternal ICR1 causes overexpression of *IGF2* in neonates and displays BWS

phenotype. Overall, overgrowth and predisposition to tumors in BWS patients is due to the loss of *CDKN1C* or overexpression of *IGF2* [129]. Methylation status of both ICR1 and ICR2 has been used to diagnose BWS and SRS prenatally [130, 131].

Prader–Willi syndrome

Prader–Willi syndrome (PWS) is a rare neurodevelopmental disorder characterized by hypotonia, short stature, delayed cognitive development, and behavioral complications. PWS is one of the leading genetic causes of pediatric obesity since PWS patients have insatiable appetite with concurrent hyperphagia. PWS has low incidence rate worldwide, i.e., about one in every 10,000 to 25,000 live birth [132, 133]. PWS results from the loss of expression of the paternally imprinted genes on chromosome 15q11.2–q13. The major mechanisms involved in PWS pathogenesis are 15q11.2–q13 microdeletions on the paternal allele (67–75% of PWS patients) and maternal UPD (25–30% of PWS patients). Epimutations in chromosome 15q11.2–q13 is rare in PWS and accounts only for 1% of PWS cases [66, 134].

On chromosome 15q11.2–q13, there are two adjacent ICRs, so-called Prader–Willi syndrome (PWS) ICR and

Fig. 2 Imprinting at the *H19-IGF2* locus and the *CDKN1C/KCNQ1* locus. **a** On maternal allele, ICR1 is unmethylated while ICR2 is methylated. Therefore, *H19*, *KCNQ1*, and *CDKN1C* are maternally expressed, whereas *IGF2* and *KCNQ1OT1* are silenced. In contrast, ICR1 is methylated and ICR2 is unmethylated on the paternal allele.

Therefore, *IGF2* and *KCNQ1OT1* are paternally expressed while other genes are silenced. **b** Beckwith–Wiedemann syndrome (BWS) occurs due to paternal UPD11 and epimutations of ICR1 or ICR2. Hence, the maternal allele act as the paternal allele with subsequent *IGF2* overexpression or loss of *CDKN1C* expression

Angelman syndrome (AS) ICR. AS/PWS ICRs control imprinting process in this *SNURF/SNRPN* imprinted cluster [135, 136]. Methylation of AS/PWS ICRs plays a very crucial role in either silencing or triggering the expression of small nuclear ribonucleoprotein polypeptide N (*SNRPN*) and the adjacent small nucleolar RNAs (*snoRNAs*) on the same allele (Fig. 3) [137]. *SNRPN* encodes a protein that is essential for the formation of spliceosomes, which are responsible for alternative splicing of different mRNAs [138].

Ubiquitin protein ligase E3A (*UBE3A*) is another important gene in *SNURF/SNRPN* cluster, which is downstream to *SNRPN* and *snoRNAs*. *UBE3A* encodes E3 ligase, which is involved in the ubiquitination of targeted proteins and ubiquitin/proteasome signaling. Imprinting of *UBE3A* is only

observed in the brain to express a well-adjusted level of E3 ligase to maintain the critical function of neurons and the typical synaptic development [139]. Therefore, deletion or overexpression of *UBE3A* results in aberrant dendritic networks, irregular synaptic connections, and abnormal levels of neurotransmitters in *Drosophila* and mice models [140, 141].

Normally, AS/PWS ICRs are both unmethylated on the paternal allele permitting the paternal expression of *SNRPN* and *snoRNAs* with silencing the paternal expression of *UBE3A*. In contrast, PWS ICRs on the maternal allele is differentially methylated resulting in absent maternal expression of *SNRPN* and *snoRNAs* with subsequent triggering expression of the maternal *UBE3A*. Inappropriate silencing of

Fig. 3 Imprinting at the *SNURF/SNRPN* cluster. AS/PWS ICRs regulate imprinting in *SNURF/SNRPN* cluster. In most tissues, *UBE3A* is biallelically expressed, whereas *UBE3A* in the brain is only expressed from the maternal side. Typically, PWS ICR is methylated on the maternal allele while it is unmethylated on the paternal allele.

Chromosomal deletions, UPD, *UBE3A* mutations, and aberrant DNA methylation at AS/PWS ICRs are the major mechanisms behind developing Angelman syndrome (AS) and Prader–Willi syndrome (PWS)

a Most tissues

b Kidney

Fig. 4 Imprinting at the *GNAS* locus. Gs-alpha is only expressed from the maternal allele in the renal tubules while it is biallelically expressed from most tissues

paternal allele or maternal UPD of *SNURF/SNRPN* cluster results in loss of expression of *SNRPN*, *snoRNAs*, and other genes. Failure to express these genes leads to the development of PWS [142–144].

Angelman syndrome

Angelman syndrome (AS) is a complex genomic imprinting disorder characterized by developmental delay, mental retardation, ataxia (movement disorder), and seizures. However, the most distinctive feature of AS is the behavioral symptoms such as paroxysmal laughter, excitable personality, hyperactivity, and happy facial appearance. Therefore, AS is also referred to as happy puppet syndrome [145]. AS has very low incidence rate, i.e., about 1 in every 12,000 to 24,000 live birth [146, 147]. AS is caused by the absence of expression of maternal *UBE3A* from *SNURF/SNRPN* cluster on chromosome 15q11.2–q13 [148, 149].

As previously described in PWS, AS/PWS ICRs regulate imprinting in *SNURF/SNRPN* cluster. In the brain, paternal expression of *SNRPN* and *snoRNAs* warrants a well-balanced expression of *UBE3A* only from the maternal allele, which ensures typical cognitive functions and normal development of the brain [150]. The primary mechanisms involved in AS pathogenesis are chromosomal deletions in maternal allele (65–75% of AS cases), paternal UPD (7%), and genetic mutations in *UBE3A* (11%). AS is rarely caused by epigenetic defect or DNA methylation aberration in AS/PWS ICRs (only 2–5% of AS cases) [151]. Quantification of DNA

methylation at *SNRPN* locus can represent a sensitive and specific technique for screening and diagnosing PWS and AS in numerous cases [152].

Pseudohypoparathyroidism type 1b

Pseudohypoparathyroidism (PHP) is an extremely rare group of genetic disorders, which result from resistance to parathyroid hormone (PTH). Increased serum PTH, hypocalcaemia, and hyperphosphatemia are the major biochemical features of PHP. PHP type 1b (PHP1b) is a subtype of PHP which displays PTH resistance only in the renal tissue. Most cases of PHP1b do not show the clinical features of Albright hereditary osteodystrophy (AHO) while AHO is dominant in PHP1a [153]. PHP1b is considered an imprinting disorder caused by defect or epimutation in ICRs which control the imprinting of *GNAS* locus on chromosome 20q13.3 [77, 82]. *GNAS* locus is an intricate imprinted locus, which encodes four transcripts (i.e., Gs-alpha, *NESP55*, A/B transcript, and *XLAS*) and the antisense *NESPAS* by alternative mRNA splicing or using different promoters and first exons [8, 154]. Imprinting of *GNAS* locus is controlled by three distinct ICRs or DMRs that are next to the promoters of *exon 1A*, *XLAS*, and *NESP55*. When these ICRs are methylated, the corresponding promoters are repressed and genes expression silenced. Therefore, *NESP55* is maternally expressed due to non-methylated ICR of *NESP55* promoter on the maternal allele (Fig. 4) [155]. Similarly, A/B transcript, *XLAS*, and *NESPAS* are paternally expressed in most tissues. *GNAS* locus expresses Gs-

Fig. 5 Loss of imprinting at the *GNAS* locus resulting in pseudohypoparathyroidism type 1b (PHP1b). In addition, maternally inherited microdeletions at the *STX16* locus are associated with phenotypic PHP1b

alpha by using *exons 1–13* whereas it can alternatively express A/B transcript by using *exon 1A* (also referred to as *exon A/B*) as a substitute first exon. A/B transcript acts in *cis* as a negative regulator to Gs-alpha expression tuning the tissue-specific imprinting of Gs-alpha particularly in the renal tubules [156].

The stimulatory G protein alpha-subunit (Gs-alpha) is one of *GNAS* domain transcripts, which is of vital importance in cell signaling cascades. When a specific ligand binds to G protein-coupled receptor (GPCR), this activates Gs-alpha that in turn triggers cAMP-dependent pathway [157]. Parathyroid hormone 1 receptor (PTH1R) is a member of the GPCR family, which requires Gs-alpha for signal transduction and physiological response to PTH [158]. Biallelic expression of Gs-alpha is observed in most cell lines and body tissues. However, Gs-alpha shows prominently tissue-specific imprinting controlled by the ICR of *exon 1A* where Gs-alpha is only maternally imprinted in the kidney, pituitary gland, and gonads. In the kidney, ICR of *exon 1A* on the maternal allele is methylated while this ICR of the paternal allele is non-methylated. DNA methylation of this ICR inhibits the expression of the A/B transcript with the subsequent typical expression of Gs-alpha. Hence, Gs-alpha is maternally expressed while the A/B transcript is paternally expressed [159, 160]. In PHP1b, DNA methylation defect in ICRs and loss of imprinting result in A/B transcript expression from the maternal allele, which suppresses the maternal Gs-alpha expression (Fig. 5). As a result, decreased or absent expression of Gs-alpha in the renal tubules confers renal resistance to PTH with subsequent biochemical changes, for example, hypocalcaemia and hyperphosphatemia. Furthermore, recent reports showed

that maternally inherited microdeletions in *STX16* locus, which is not imprinted locus and exists upstream to *GNAS* locus, are associated with the loss of imprinting in *GNAS* locus displaying PHP1b phenotype [76, 161, 162].

Conclusion

Genomic imprinting is an epigenetic phenomenon that is involved in normal growth, viability, and embryonic development. Only small fraction of genes within the whole genome undergoes imprinting. These imprinted genes are typically organized in clusters controlled by imprinting control regions. The expression of these genes occurs in a parent-of-origin-specific manner. Therefore, imprinted genes are expressed only from one parent, while the other parent's copies of these genes are silenced. However, tissue-specific expression of some imprinted genes was reported due to the complexity of genomic imprinting. DNA methylation and histone modifications play the key role in the process of imprinting.

Imprinting clusters are susceptible to several mutations, deletions, and epigenetic aberrations that result in loss of imprinting and imprinting disorders. Besides, the extensive use of assisted reproductive technology (ART) in conception is associated with an increased incidence of imprinting disorders. Imprinting disorders are complex syndromes of neurodevelopmental disabilities, cognitive problems, and metabolic diseases. DNA sequencing and DNA methylation analysis of ICRs/DMRs in different clusters represent a standard method for diagnosing imprinting disorders.

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