Review

DNA Methylation Dynamics in the Female Germline and Maternal-Effect Mutations That Disrupt Genomic Imprinting

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Abstract: Genomic imprinting is an epigenetic marking process that results in the monoallelic expression of a subset of genes. Many of these 'imprinted' genes in mice and humans are involved in embryonic and extraembryonic growth and development, and some have life-long impacts on metabolism. During mammalian development, the genome undergoes waves of (re)programming of DNA methylation and other epigenetic marks. Disturbances in these events can cause imprinting disorders and compromise development. Multi-locus imprinting disturbance (MLID) is a condition by which imprinting defects touch more than one locus. Although most cases with MLID present with clinical features characteristic of one imprinting disorder. Imprinting defects also occur in 'molar' pregnancies-which are characterized by highly compromised embryonic development-and in other forms of reproductive compromise presenting clinically as infertility or early pregnancy loss. Pathogenic variants in some of the genes encoding proteins of the subcortical maternal complex (SCMC), a multi-protein complex in the mammalian oocyte, are responsible for a rare subgroup ofdother f(

Citation: Anvar, Z.; Chakchouk, I.; Demond, H.; Sharif, M.; Kelsey, G.; Van den Veyver, I.B. DNA Methylation Dynamics in the Female Germline and Maternal-Effect Mutations That Disrupt Genomic Imprinting. *Genes* **2021**, *12*, 1214. https://doi.org/10.3390/genes12081214

Academic Editor: Miguel Constancia

Received: 15 June 2021
Accepted: 3 August 2021
Published: 6 August 2021

Following fertilization, the genome of the mouse zygote undergoes another reprogramming wave (Figure 1). The timing and mode of reprogramming differ between the

Until week 5, the genomes of PGCs are highly methylated (Figure 1), but must be reprogrammed to give rise to functional gametes. DNA methylation reprogramming at this stage is concomitant with the downregulation of DNMT3A, DNMT3B, and UHRF1, the DNMT1 accessory factor, and the upregulation of TET1 and TET2 [31]. The lowest DNA methylation level in the human genome is achieved after this reprogramming at 10–11 weeks pf. At this stage, global DNA methylation levels were reported to be 8% in male PGCs at 11 weeks and 6% in female PGCs at 10 weeks (Figure 1) [32,33].

The timing of methylation establishment during oogenesis in humans has recently

stored proteins and RNAs that are expressed from the maternal genome in the developing oocyte. The genes from which these RNAs are expressed are referred to as maternal-effect genes as they are transcribed from the maternal genome before fertilization, but their products are essential for the developing embryo until its genome is transcribed. After fertilization, the maternally provided transcripts and proteins gradually degrade while ZGA (which is completed at the four to the eight-cell stage in human embryos and at the two-cell stage in mouse embryos) encompasses the beginning of embryonic transcription, which will gradually take over control of processes, such as cell differentiation and future development [44–46].

DNA demethylation in the human preimplantation embryo occurs in a stepwise manner reaching a mean DNA methylation level of 25.7% in blastocyst-stage embryos at five to six days pf [13,19]. This is significantly lower than the median DNA methylation levels in the egg of 54.5% and sperm of 82%, indicating a significant reduction from the highly specialized gametes to the totipotent embryo [13,19]. Generally, the oocyte hypermethylated domains maintain intermediate methylation levels in the blastocyst [47], but they respond differently to global demethylation based on their genomic properties. For example, transposable elements which are hypermethylated in oocytes are drastically demethylated in blastocysts, with the exception of SINE-VNTR-Alu (SVA) and LTR12 subfamilies, which retain high levels of residual methylation throughout the preimplantation period [13,48]. More so than in mouse embryos, highly methylated CGIs retain substantial levels of methylation in human embryos (median of methylation of these CGIs and gDMRs in the blastocyst is 37.5% and 39.2%, respectively) [13]. The maternal genome is less demethylated in humans than in mouse embryos during preimplantation development. Consequently, the global methylation levels of the human blastocyst closely resemble those of the oocyte [47].

Demethylation of the paternal genome is much faster and more profound than that of the maternal genome in human embryos, which is more similar to the same process in the mouse, suggesting that active demethylation is conserved between the two species [38,48]. Accordingly, the residual DNA methylation levels, in either male pronuclei or the paternal genome domains from the 2-cell stage onward are always lower compared to those of the maternal genome [19,38].

In addition to genome-wide demethylation events, limited de novo methylation of active repeat elements during preimplantation development in human embryos has recently been reported [19]. In particular, SINEs, LINEs, and long terminal repeats (LTRs)

originates from ICM-derived extraembryonic mesoderm [54]. As mentioned above, during post-implantation development the genome acquires de novo methylation. However, the placenta maintains a general hypomethylated epigenome compared to the embryo [55]. The placental methylome is organized into partially methylated domains (PMD) and highly methylated domains (HMD) [56]. PMDs are large domains covering about 40% of the genome with an average DNA methylation level of 45% (compared to 80% in HMDs) and overall lower transcription levels than the rest of the genome. The placenta is the only

lifelong memory of parental origin into the next generation (Figure 1) [69,70]. In humans, some gDMRs are maintained exclusively in the placenta. This phenomenon is called placenta-specific imprinting and has not yet been observed in the mouse [47,54,71–74]. Most placenta-specific imprinted genes are transient and retain methylation of maternal origin set in the oocyte [54,71]. Placenta-specific imprinted genes show mono-allelic methylation on the maternal allele in placental villi, cytotrophoblasts, trophoblast, and mesenchyme mostly become unmethylated in somatic tissues [54,71]. The incomplete demethylation of the maternal allele during preimplantation development or incomplete de novo methylation of the paternal allele post-implantation are proposed as mechanisms of placenta-specific imprinting [47].

4. Global Loss of Imprinting Results in Hydatidiform Molar Pregnancies

The two waves of DNA methylation reprogramming in the germline and the early embryo are important for normal development. In particular, the establishment and maintenance of imprints have been shown to be crucial for the maintenance of a healthy pregnancy. Therefore, the loss of imprinting results in a variety of developmental abnormalities [21,75]. The most severe form is the hydatidiform mole (HM), which is a gestational abnormality characterized by trophoblast overgrowth and the absence of embryo development [76]. In most cases, HM pregnancies occur sporadically and are the result of an androgenetic embryo that has two paternal genome copies and is lacking the maternal copy. The lack of the maternal copy consequently means that all maternal imprints are missing, while the paternal imprints are fully methylated, which is thought to be the main factor driving the HM phenotype. In mice, androgenetic pregnancies lacking maternal imprints show similarities to the HM phenotype in that they are characterized by trophoblast overgrowth and abnormal development of the embryo proper [77,78]. The lack of imprinting results in the imbalance of imprinted gene expression. In a recent study using bipaternal mice, this imbalance of expression was corrected at seven imprinted loci, which resulted in the birth of live pups, highlighting the importance of mono-allelic expression of imprinted genes for normal pregnancies [79].

In rare cases, HM are recurrent, and in most such instances, this coincides with a biparental genome. These are termed biparental complete hydatidiform mole (BiCHM). The majority of BiCHM pregnancies have been associated with mutations in the maternal-effect genes *NLRP7* (~75%) and *KHDC3L* (~5–10%) [25]. Recently, a patient with a *PADI6* mutation was identified [80]. In contrast to androgenetic HMs, BiCHM has a maternal copy of the genome. Their phenotype is associated with widespread loss of methylation at (almost) all maternal gDMRs in patients with disease variants in *NLRP7* and *KHDC3L* [22,81–84]. Given that only the maternal and not paternal gDMRs appear to be affected, it was suggested that the loss of methylation originates in the oocyte. Indeed, a recent study assessing DNA methylation in oocytes of a patient with a *KHDC3L* mutation showed that global DNA

and regulates several essential cellular processes during the egg-to-embryo transition, such as spindle assembly, chromosome alignment, and symmetric cell division in cleavage-stage embryos [86,87]. The SCMC has since also been detected in other mammalian species, including humans [85,88]. In humans, seven genes have, so far, been described to encode proteins of the SCMC: *NLRP5* (*MATER*), *OOEP* (*FLOPED*), *TLE6*, *NLRP2*, *NLRP7*, *KHDC3L* (*C6ORF221*), and *PADI6* [85,89–91].

The SCMC is localized in the subcortical region of the cytoplasm just below the cell membrane or cortex of the oocyte and persists throughout preimplantation development until the blastocyst stage, where it is excluded from regions with cell-cell contact. The cytoplasmic localization of the SCMC makes a potential role in imprinting regulation all the more intriguing. As discussed above, mutations in *NLRP7*, *KHDC3L*, and, potentially, *PADI6* are associated with BiCHM pregnancies. Other genes, including *NLRP5*, *NLRP2*, *NLRP7*, *PADI6*, and *OOEP*, have been associated with MLID and miscarriages [22,27,28,92]. MLID is thought to occur from failure of gDMR maintenance in preimplantation embryos because, in contrast to BiCHM, only a variable subset of gDMRs is affected in MLID. The cause of the high frequency of miscarriages in some women with SCMC mutations is unknown [22,27,28]. One possibility is that the number of imprinted genes affected varies between offspring, and only milder cases develop to term. Another gene frequently mutated in MLID is *ZFP57* [66] which, together with *DNMT1* and *TRIM28*, encodes part of the DNA methylation maintenance machinery of gDMRs during preimplantation development [93]. How the SCMC members impact the maintenance machinery remains to be resolved. One possibility is that the SCMC functions as a regulator of cellular organization and through that can regulate the localization of proteins involved in DNA methylation, such as DNMTs. Indeed, knockout of *Nlrp2* in mice disrupts the subcellular localization of DNMT1, but not DNMT3A [94]. Immunofluorescence showed that DNMT1, which was enriched in the cortex together with other SCMC proteins in control oocytes and preimplantation embryos, had a more diffuse cytoplasmic rather than cortical localization in maternal knockout zygotes [94]. This would suggest an involvement of NLRP2 in DNA methylation maintenance, which fits with the association of NLRP2 with MLID in humans [28,92]. Mid-gestation embryos and neonates from *Nlrp2*Nl.038 0 1 0.71765 rg 0.0 0 1 16

independent of imprinting disorders [100–104]. It has been proposed that there might be a causal link between the SCMC, DNA methylation, and genome integrity, as imprinting aberrations have been associated with aneuploidies in the embryos of patients with SCMC mutations [21,22,28,75]. A role for the SCMC in ploidy is also supported by a mouse study, in which maternal ablation of *Khdc3* caused abnormal spindle assembly, chromosome misalignment, and spindle assembly checkpoint inactivation during the early embryo

Table 1. Summary of familial and singleton variants within SCMC genes causing early embryonic lethality, MLID, and BiCHM.

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
NALP7	MoLb1	Chr19: 55452298	NM_206828	IVS3+1G>A	2 splicing isoforms: -inclusion of the first 4 bp of intron 3 between exons 3 and 4, addition of two aa followed by a stop codon -exclusion of exon 3	Splicing mutation (Splice donor)	0.00E + 00	0.00E + 00	NA	NA	Autosomal recessive (Homozy-gous)	Intron 3	Recurrent hydatidi-form moles	Lebanon	[25]
	MoPa61	Chr19: 55445856		IVS7+1G>A	inclusion of the entire intron 7	Splicing mutation	5.17E-05	NA	NA	NA	Autosomal recessive (Homozy-gous)	Intron 7	Complete hydatidi-form mole, sponta-neous abortion (7–20 weeks)	Pakistan	
	MoGe2	Chr19: 55449464		2077C>T	p.Arg693Trp	Missense mutation	2.74E-04	6.69E-04	NA	NA	Autosomal recessive (Homozy-gous)	Exon 5	Complete hydatidi-form mole	Germany	
	MoIn68	Chr19: 55449463		2078G>C	p.Arg693Pro	Missense mutation	4.77E-05	NA	tolerated (0.07)	benign (0.056)	Autosomal recessive (Homozy-gous)	Exon 5	Complete hydatidi-form mole	India	

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	MoIn69-2	Chr19: 55441939		c.2738A>G	p.Asn913Ser	Missense mutation	1.35E-04	7.33E-04	deleterious (0)	probably_ damag- ing (0.991)					

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Patient 1 and 2	Chr19: 55449463	NM_001127255.1	c. 2078G>C	p.Arg693Pro	Missense mutation	4.77E-05	NA	tolerated (0.07)	benign (0.056)	Autosomal recessive	Exon 5	Complete hydatidi-form moles	UK	[84]
	Patient 3	Chr19: 55449184_ 55454887 del		c.-39-1769_2129+ 228del		Deletion of exons 2-5	NA	NA	NA	NA	Autosomal recessive	5^{0}UTR			
	Patient 4	Chr19: 55449523		c.2018C>G	p.Ser673Ter	Nonsense mutation	3.98E-06	NA	NA	NA	Compound Heterozy-gous	Exon 5			
		Chr19: 55447768		c.2161C>T	p.Arg721Trp	Missense mutation	5.97E-05	NA	NA	NA		Exon 6			
	Family E	Chr19: 55451235_ 55451248	NM_206828.3	c.939_952 dup 14	p.Tyr318Cys fsTer7	Frameshift mutation	2.39E-05	1.27E-04	NA	NA	Compound Heterozy-gous	Exon 4	Familial biparental hydatidi-form mole	UK	[106]
		Chr19: 55449511		c.2030delT	p.Leu677Pro fsTer6										

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Family K	Chr19: 55449463		c.2078G>C	p.Arg693Pro	Missense mutation	4.77E-05	NA							

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	MoCh200	Chr19: 55450487_55450562 del76		c.1625_1700 del76	p.Met542ThrfsTer2	Frameshift mutation	NA	NA	NA	NA	Compound Heterozy-gous	Exon 4	HM		
		Chr19: 55445108		c. 2471+1G>A	p.Leu825Ter	Nonsense mutation	NA	NA	NA	NA		Exon 7			
	MoCh293	Chr19: 55450893		c.1294C>T	p.Arg432Ter	Nonsense mutation	3.61E-05	3.19E-05	NA	NA	Compound Heterozy-gous	Exon 4	HM		
		Chr19: 55447773		c.2156C>T	p.Ala719Val	Missense mutation	1.05E-03	1.05E-03	deleterious (0.01)	probably_ damaging (0.963)		Exon 6			
	MoCh73	Chr19: 55451050		c.1137G>C	p.Lys379Asn	Missense mutation	5.01E-03	6.08E-03	NA	NA	Heterozygous	Exon 4	CHM		
	MoCh71	Chr19: 55452829		c.251G>A	p.Cys84Tyr	Missense mutation	4.53E-04	3.20E-04	tolerated (0.05)	benign (0.079)	Heterozygous	Exon 2	AnCHM		
	MoCh193	Chr19: 55451050		c.1137G>C	p.Lys379Asn	Missense mutation	5.01E-03	6.08E-03	NA	NA	Heterozygous	Exon 4	HM		
	MoCh190	Chr19: 55445860		c.2468T>A	p.Leu823Ter	Nonsense mutation	NA	NA	NA	NA	Heterozygous	Exon 7	AnCHM		

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	MoCh71	Chr19: 55452829	NM_001127255.1	c.251G>A	p.Cys84Tyr	Missense Mutation	4.53E-04	3.20E-04	tolerated (0.05)	benign (0.079)	Heterozygous	Exon 2	CHM, PHM (with no family history of moles)	China	[105

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Ch101	Chr19: 55449440		c.2101C>T	p.Arg701Cys	Missense mutations	1.99E-05	NA	tolerated (1)	benign (0.018)	Compound Heterozy-gous	Exon 5	CHM (with no family history of moles)	China	
		Chr19: 55449463		c.2078G>A	p.Arg693Gln		7.95E-06	3.19E-05	tolerated (0.07)	benign (0.056)					
	MoCa94	Chr19: 55447773		c.2156C>T	p.Ala719Val	Missense mutation	1.05E-03	1.05E-03	deleterious (0.01)	probably_ damaging (0.963)	Heterozygous	Exon 6	PHM (with no family history of moles)	Italy	
	Ch29	Chr19: 55447764		c.2165A>G	p.Asp722Gly	Missense mutation	3.98E-06	NA	deleterious (0.05)	possibly_ damaging (0.574)	Autosomal recessive (Homozy-gous)	Exon 6	PHM, BiCHM, CHM (with no family history of moles)	China	
	MoUs99	Chr19: 55447681		c.2248C>G	p.Leu750Val	Missense mutation	5.29E-04	9.56E-05	NA	NA		Exon 5	PHM, CHM, HM (Familial recurrent HMs)	Mexico	
	Ch77	Chr19: 55450893		c.1294C>T	p.Arg432Ter	Nonsense mutations	3.61E-05	3.19E-05	NA	NA	Compound Heterozy-gous	Exon 4	CHM	China	
		Chr19: 55445108		c.2471+1 G>A	p.Leu825Ter		NA	NA	NA	NA		Intron 7			
	MoFr101	Chr19: 55439063		c.2891T>C	p.Leu964Pro	Missense mutation	NA	NA	deleterious (0)	probably_ damaging (1)	Autosomal recessive (Homozy-gous)	Exon 10	PHM	France	

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
NLRP5	Family 1	Chr19: 56544020	NM_153447.4	c.2320T>C											

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Family 7	Chr19: 56538465		c.866G>A	p.Gly289Glu	Missense mutation	NA	NA	deleterious (0)	probably_ damaging (1)	Compound Heterozy-gous	NACHT			
		Chr19: 56569626		c.3320C>T	p.Thr1107Ile	Missense mutation	NA	3.19E-05	deleterious (0)	probably_ damaging (0.993)		LRR			
	Family 1	Chr19: 56538660		c.1061C>T	p.Pro354Leu	Missense mutation	1.21E-05	3.19E-05	deleterious (0.03)	probably_ damaging (0.999)	Autosomal recessive	NACHT	Recurrent early embryonic arrest	China	[107]
NLRP2	Family 1	Chr19: 55494543	NM_017852.4	c.1479_1480 delAG	p.Arg493Ser fsTer32	Frameshift mutation	7.56E-05	NA	NA	NA	Autosomal recessive (Homozy-gous Mother), Heterozy-gous in both probands	LRR domain	MLID	Germany	[28]
	Family										Autosomal recessive consan-guineous family		Proband with BWS–MLID	Pakistan	[92]

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Family 2	Chr19: 55497553		c.2237delA	p.Asn746ThrfsTer4	Frameshift mutation	3.98E-06	NA	NA	NA	Heterozygous mother and proband	Exon 8	Proband with SRS	Germany	Family previously reported in [108] and [109]
	Family 3	Chr19: 55505788		c.2860_2861delTG	p.Cys954GlnfsTer18	Frameshift mutation	NA	NA	NA	NA	Heterozygous mother	Exon 11 / LRR domain	Proband 47, XXY, Symmetrical growth restriction and developmental delay	Germany	[28]
	Family 4	Chr19: 55485901		c.314C>T	p.Pro105Leu	Missense mutation	2.79E-05	NA	tolerated (0.15)	possibly_ damaging (0.604)	Heterozygous mother	Exon 3	TNDM		[28]
	Family 5	Chr19: 55494951		c.1885T>C	p.Ser629Pro	Missense mutations	1.01E-03	1.12E-03	deleterious (0)	probably_ damaging (0.959)	Compound Heterozygous (Mother and Proband)	Exon 6	SRS	UK	[28]
		Chr19: 55501424		c. 2401G>A	p. Ala801Thr		9.17E-03	1.27E-02	tolerated (0.51)	benign (0.097)		Exon 9			

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
NM_017852.5	Family 1	Chr19: 55495027		c.1961C>A	p.Ser654Ter	Nonsense mutation	NA	NA	NA	NA	Autosomal recessive	Exon 6	MLID	China	[104]
	Family 2	Chr19: 55493839		c.773T>C	p.Phe258Ser	Missense mutation	3.98E-06	NA	deleterious (0)	probably_ damag-			Outcomes		

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
KHDC3L	Family L	Chr6: 74072455	NM_001017361.3	c.3G>T	p.Met1Ile next available downstream ATG codon lies at residue 14	Loss of start codon	3.98E-06	NA	deleterious (0)	probably_ damaging (0.916)	Autosomal recessive (consan-guineous family)	Exon1	Familial Biparental Hydatidi-form Mole	Pakistan	[26]
	Family T	Chr6: 74072970		c.322_325 delGACT	p.Asp108Ile fsTer30	Frameshift mutation	2.39E-05	NA	NA	NA		Exon 2	Complete Hydatidi-form Mole	Tunisia	
	Family W	Chr6: 74072453													

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Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Family 1	Chr1: 17718714		c.1067G>A	p.Trp356Ter	Nonsense mutation	NA	NA	NA	NA	Probands mother is Com-pound Heterozy-gous	PAD domain (Exon 10)	Beckwith-Wiedemann syndrome with multi-locus imprinting distur-bance	Italy	[110]
		Chr1: 17727743		c.1894C>G	p.Pro632Ala	Missense mutation	4.01E-06	NA	deleterious (0)	probably_ damag-ing (1)		PAD domain (Exon 17)			
	Family 2														

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
	Family 10	Chr1: 17394024		c.1124T>C	p.Leu375Ser	Missense mutations	NA	NA	deleterious (0.01)	probably_ damaging (0.915)	Compound Heterozy-gous (Mother)	Exon 10	BWS–MLID		
		Chr1: 17397091		c.1639G>A	p.Asp547Asn		NA	5.06E-04	tolerated (1)	benign (0.005)	Heterozygous in Proband	Exon 14			
	Family 11	Chr1: 17392197		c.1046A>G	p.Asp349Gly	Missense mutation	NA	NA	tolerated (0.37)	probably_ damaging (0.953)	Heterozygous (Mother)	Exon 9	SRS	Germany	
	Family 12	Chr1: 17379985		c.433A>G	p.Lys145Glu	Missense mutation	NA	6.57E-06	deleterious (0.02)	possibly_ damaging (0.612)	Heterozygous (Mother)	Exon 4	SRS	Germany	
OOEP (hg38)	Family 13	Chr6: 73369684	NM_001080507.2	c.109C>T	p.Arg37Trp	Missense mutation	NA	3.29E-05	deleterious (0.04)	benign (0.135)	Autosomal recessive (Homozy-gous Mother), Heterozy-gous proband	Exon 1	TNDM		
UHRF1 (hg38)	Family 14	Chr19: 4930782	NM_013282.4	c.514G>A	p.Val172Met	Missense mutation	NA	NA	deleterious (0)	probably_ damaging (0.952)	Heterozygous (Mother and Proband)	Exon 3	SRS		

Table 1. *Cont.*

Gene	Family	hg19 Position	GenBank	cDNA Mutation	Protein Mutation	Mutation Effect	gnomAD_ Exomeall MAF	gnomAD_ Genomeall MAF	SIFT	Polyphen	Inheritance	Domain/ Exon	Pregnancy Outcomes	Country	Ref
ZAP2Z3															

DAPIN (Domain in Apoptosis and Interferon response) domain. Protein arginine

by grants (R01HD079442 and R01HD092746) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health, by cores from the Intellectual and Developmental Disabilities Research Center (IDDRC; grant P50 HD103555). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. M.S. is supported by postdoctoral fellowship award from The Lalor Foundation, INC.

Conflicts of Interest: The authors declare no conflict of interest.

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