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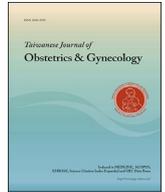
Review Article

Clinical and genetic-epignetic aspects of recurrent hydatidiform mole: A review of literature

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ABSTRACT

Hydatidiform Mole (HM) is the most common form of Gestational Trophoblastic Disease (GTD), defined by hyper-proliferation of trophoblastic cells. HM is typified as abnormal proliferation of extraembryonic trophoblastic (placental) tissues and failure of embryonic tissues development and is the only GTD with Mendelian inheritance, which can reoccur in different pregnancies. Moles are categorized into Complete Hydatidiform Moles (CHM) or Partial Hydatidiform Moles (PHM) and a rare familial trait, which forms a CHM and despite having androgenetic pattern, shows normal biparental inheritance, conceived from one sperm and egg. Recessive maternal-effect mutations in NLRP7 (NACHT, leucine rich repeat and PYD containing 7) and KHDC3L (KH Domain Containing 3-Like) genes have been shown to be responsible for Recurrent Hydatidiform Moles (HYDM1 MIM# 231090 when is caused by mutation in the NLRP7 gene and HYDM2 MIM#614293 when is caused by mutation in the KHDC3L gene). Methylation aberration in multiple maternally imprinted genes is introduced as the cause of Recurrent HYDM pathology. The current article reviews the histopathology, risk factors, and genetic and epigenetic characteristics of Recurrent HYDMs.

Epidemiology and risk factors

Incidence

The incidence of gestational trophoblastic disease is both geographic and ethnic-related. Due to high incidence of molar pregnancy in some populations, studies have associated low socio economic status with high incidence of GTD [1]. GTD incidence is three to four times higher in Asia, Africa and Latin America than in North America and Europe. GTD incidence has remained relatively constant at 1 to 2 per 1000 deliveries in Europe and in United States [2]. However, despite substantial economic achievements over the recent years, Japan yet shows a relatively high frequency (3 in 2000 deliveries in 2000 and 1/500 pregnancies in 2003) of molar pregnancy. On the other hand, GTD occurs in a rate of 28 per 1000, 8.5 per 1000, 9.8 per 1000 and 2 per 1000 in Pakistan, Brazil, Finland and Sweden respectively [2,3]. As documented, Hispanics and Native Americans residing in the United States and certain population groups in South East Asia show a higher incidence of molar pregnancy compared to the rest of the population living in the same countries 8 [4]. Considering the global statistics, genetic, nutritional and environmental factors also seem to play roles in GTD development [3]. The incidence of Hydatidiform Mole in Hamadan in west of Iran was estimated 3.34 per 1000 pregnancies between 1997 and 2006. Among the cases with mole, 53.29% were complete and 46.71% were partial mole [5].

Risk factors

Clinical studies have been carried out to identify risk factors for molar pregnancy and discover whether factors differ in CHM or PHM. Maternal age at upper and lower extremes, i.e. teenage women and those aged over 35 have 2–3 fold increased risk of

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developing complete molar pregnancy [2]. This risk escalates up to 7 fold for women older than 40 years, which could be attributed to higher susceptibility of ovum from old women to abnormal fertilization. Old paternal age, history of spontaneous abortion or previous gestational trophoblastic disease, low dietary intake of carotene and vitamin A deficiency [6], certain ABO blood groups and smoking have been reported to carry a higher risk of CHM development. On the other hand, there is limited knowledge concerning risk factors for partial molar pregnancy. Oral contraceptive use and history of irregular menstruation have been linked to increased risk of PHM development. However, no association between maternal or dietary intake with partial mole has been reported [6]. Recurrent HYDM is a sub-class of CHM and clearly mentioned risk factors, threaten women to develop also this form. However One most significant risk factor associated with recurrent HYDM is maternal homozygous and compound mutations in maternal effect genes, *NLRP7* and C6ORF221, further discussed below [7]. Moreover, other factors including previous molar pregnancy either partial or complete, family history of molar pregnancy and maternal age over 40 has also been mentioned [1].

Clinical presentation and diagnosis

Due to current routine use of para clinical technology, the clinical presentation of molar pregnancy has been transformed drastically over the past few decades. Serum β-hCG measurement and transvaginal ultrasonography have reduced the mean gestational age of complete molar diagnosis from 16 to 17 weeks in 1960s and 1970s to 12 weeks, today. A significant feature of molar pregnancies is their overproduction of β-hCG as the consequence of trophoblastic overgrowth, resulting in markedly high levels of serum β-hCG in excess of that expected for the gestational age [8]. However, first trimester serum β-hCG levels may not always be elevated, in which cases sonography has proved to be a more useful tool for molar pregnancy diagnosis [9,10]. Sonographic appearance of CHM reveals a diffuse intrauterine complex echogenic mass with tiny cystic spaces and absent fetal tissue [9]. On the contrary PHM sonographic feature is characterized by a thickened hydropic placenta with a concomitant fetus [10].

Histopathology

Trophoblastic diseases are characterized by aberrant histological changes within placenta. Being the most common form of gestational trophoblastic disease, hydatidiform mole specifically is characterized with abnormal or absence of fetal development, excessive trophoblastic overgrowth and hydropic villous degeneration [11]. In microscopic evaluation, CHM, which represents approximately 75% of molar pregnancies involves diffuse edematous villi and trophoblastic hyperplasia in the entire placenta [12]. Macroscopically, no fetal tissue or amnion development is observed. As apparent from the term partial HM, the extent of villous edema, trophoblastic proliferation and signs and symptoms are comparatively lower than that of CHM. Furthermore, partial moles contain fetal tissue and amnion in addition to placental tissue.

Hematoxylin & eosin (H&E) staining of sections of chorionic villi from CHMs reveals the presence of excessive circumferential trophoblastic proliferation around most CVs with no embryonic tissue of inner cell mass origin such as fetal membrane, cord or nucleated blood cells. That is completely in contrast to H&E stained sections of PHMs in which mild or focal trophoblastic proliferation of some CVs along with fetal tissues and sometimes even abnormal or normal complete fetus are displayed [13].

Recurrent HYDM is a familial pathology defined by the occurrence of at least two moles in the same patient and affects 1.5—9.3%

of women with a prior HM [14]. Recurrent HYDMs mostly are CHM. Although some PHM have been described and in the rare cases the woman also had live-born offspring [13,15]. They are phenotypically like CHM of androgenetic origins (AnCHM) wherein both genomes being paternally derived. It means both parents contribute equally their genome to the formation of this kind of mole. Biparental HYDMs have familial property and can recur more times in the same individual. Clearly recurrent pattern in the case of single pregnancy is senseless and so the best candidate nomination for Recurrent HYDM is Biparental HM (BiHM) [11,12]. The absence of bias for one of two genomes in these moles and the phenotype of HMs in the same time, indicate that this pathology is linked to any deregulation in imprinted genes expression.

Genetic basis of recurrent HYDMs and responsible genes

Genetically, CHMs have diploid karyotypes, 85% of which are result of androgenesis. In androgenesis a chromosomally inactivated or enucleated ovum is fertilized by a haploid sperm, which then duplicates via meiosis producing 46XX karyotype with complete paternal origin [16] (Fig. 1-A, Modified from Williams and colleagues, 2010 [12]). In the remaining, dispermic fertilization of a single ovum results in 46XY paternal karyotype (Fig. 1-B, Modified from Williams and colleagues, 2010 [12]). Apparently 46XX karyotype never survives. PHMs in contrast, show triploid karyotypes. Most PHMs are reported to develop from dispermic fertilization of an egg showing 69XXX or less occasionally 69XYY (Fig. 1-D, Modified from Williams and colleagues, 2010 [12]). However, 69XYY also less commonly arises from fertilization of an egg by a single diploid sperm [12].

Recurrent HYDM is a sub group of CHM and despite having androgenetic phenotype shows normal biparental inheritance, conceived from one sperm and egg (Fig. 1-C, Modified from Williams and colleagues, 2010 [12]). The karyotype of these moles is 46XX or 46XY. Recessive maternal-effect mutations in *NLRP7* and *KHDC3L* (also known as C6ORF221) genes have been shown to be responsible for Recurrent HYDM [1,17]. The best method to differentiate this form of moles is genetic testing [18]. In most of women with Recurrent HYDM, homozygote or compound heterozygote mutations have been seen in *NLRP7* or *KHDC3L* genes [19].

NLRP7

Initially linkage analysis has shown that in most families the gene responsible for Recurrent HYDM is located on 1.1 Mb region on chromosome 19q13.4. Mutations in this gene result in imprinting dysregulation in the female germ line with abnormal development of both embryonic and extraembryonic tissues [20]. As women continued to have recurrent molar pregnancies with more than one partner, autosomal recessive pattern was suggested in women themselves responsible for disrupting normal oocyte fertilization with no paternal genomic involvement [12,21]. In 2006 Murdoch and colleagues identified *NLRP7* (NOD-like receptor pyrin domain (PYD)- containing 7) as the candidate maternal-effect gene responsible for Recurrent HYDM and reproductive wastage such as spontaneous abortions and stillbirths [22]. *NLRP7* is located on 19q13.42 and encodes for a protein of 1037 amino acids. *NLRP7* belongs to the CATERPILLER family of proteins and contains four conserved and functional domains consists of a N-terminal pyrine domain, 9—10 leucine-rich repeats (LRRs) depending on splice isoforms in C-terminal domain, NACHT-associated domain (NAD) (physical mediator for oligomeric assembly) and a NACHT region in the middle of the protein (this domain contain Walker A/Ploop motif which is a binding site for ATP) (Fig. 2) [23,24]. Unlike LRR and pyrine domains on *NLRP7*, which are involved in protein—protein
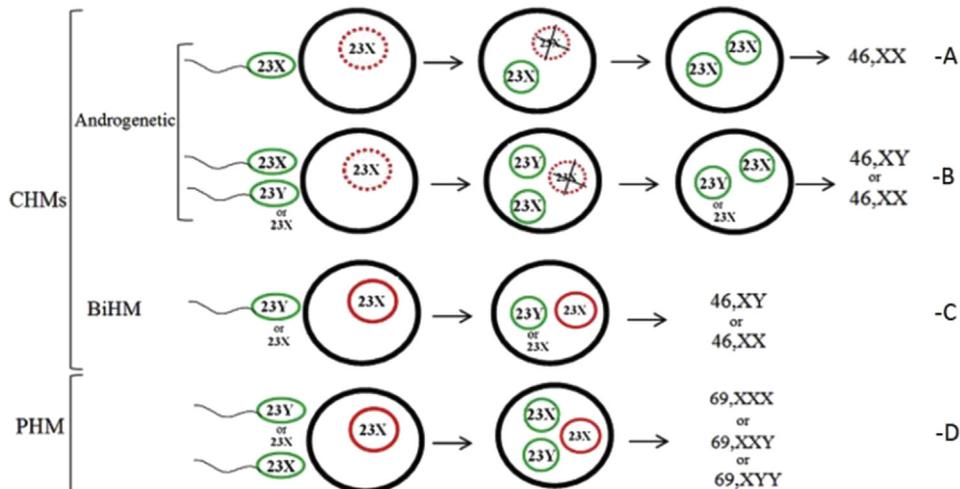
Fig. 1. Genetic abnormality causes Hydatidiform Moles (HMs). Majority of HMs are sporadic CHMs and have androgenetic origin which include A- Monospermic (duplication of the paternal haploid genome can be seen in the last stage) and B- Dispermic fertilization with an anuclear oocyte. C- In BiHM one set of each parental chromosome inherited but *NLRP7* or *KHDC3L* genes have mutated in homozygous state in mother. D- PHMs are triploid in which two set of chromosomes are inherited from father and other set from mother Modified from Williams and colleagues, 2010 [12].
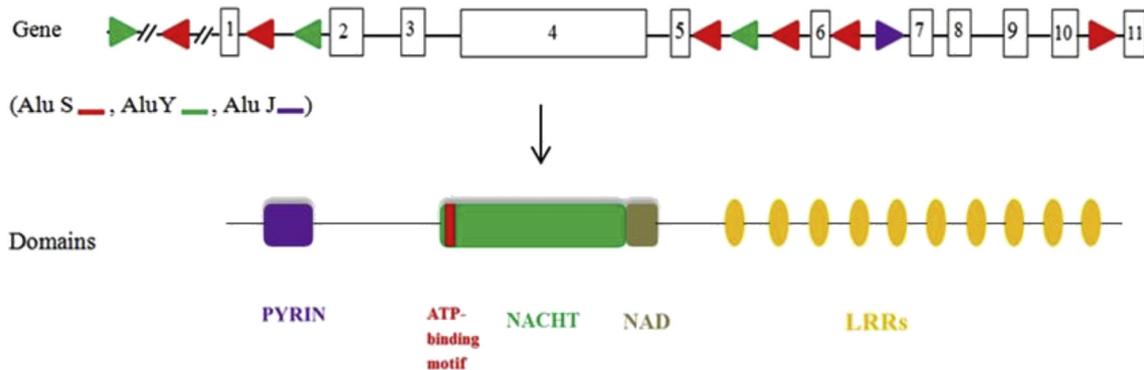

Fig. 2. *NLRP7* gene consist of exons, introns and repeat sequences. Eleven exons and the location of different Alu repeat sequences were depicted. *NLRP7* proteins contain pyrin, NACHT, NAD and leucine-rich repeats domains.

interactions, NACHT domain is involved in apoptosis as well as MHC class II transactivation [25]. Members of CATERPILLER family of proteins are widely expressed in various tissues in different species from C. elegans, D. melanogaster, rat, and mouse to human [26]. They act as inflammasome components and play roles in innate immunity, inflammation and apoptosis. Genomic sequences of *NLRP7* gene contain Alu repetitive elements, which represent about 48% of its intronic sequences and is suggested as hotspot for indel mutations, which is reported in this gene [14] (Fig. 2).

NLRP7 is mutated in 48—80% of patients with Recurrent HYDM. To date, 59 pathogenic mutations in *NLRP7* have been identified. *NLRP7* transcripts have been detected in several human tissues, such as hematopoietic cells, endometrium, all oocytes stages, placenta, and preimplantation embryos [14,18] with the highest expression level in the testis. *NLRP7* is expressed in spermatozoa and all stages of oocyte and early embryonic development with its lowest level on day 3 post fertilization and the highest expression on day 5 corresponding to blastocyst stage [13,19]. Patients with *NLRP7* mutations produce significantly lower amounts of IL-β and TNF in response to LPS, thus playing role as a part of immune system [23].

In a women with homozygous mutation in *NLRP7* gene, assisted reproductive techniques such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) into ovum accompanied by Preimplantation Genetic Diagnosis (PGD) have been used but the

satisfactorily results have not been achieved [27]. Fisher and his colleagues [28] reported a successful pregnancy from donated oocytes to women with three RHMs and compound heterozygote mutation in *NLRP7*. Recently also a successful live birth is reported from donated ovum within a mother with homozygous and compound heterozygous mutations in *NLRP7* [29].

KHDC3L

In 2011 Parry and colleagues identified *KHDC3L* on chromosome (KH domain containing 3-like) 6q13 as a new recessive gene, which is involved in 10—14% of Recurrent HYDM cases with no *NLRP7* mutations [30]. *KHDC3L* gene has three exons that encodes for a protein of 217 amino acids (Fig. 3). *KHDC3L* protein belongs to the KHDC1 (KH homology domain containing 1) protein family. Unlike other members (KHDC1, DPPA5, OOEP), *KHDC3L* has an atypical KH domain that cannot bind to RNA. *KHDC3L* transcripts have been detected in several human tissues, including hematopoietic cells, all oocytes stages, and preimplantation embryos. To date, four pathogenic mutations have been identified in *KHDC3L* gene [18].

NLRP7 and KHDC3L localization

There are many similarities in expression pattern of *NLRP7* and *KHDC3L* [19]. In protein level, immunofluorescence and confocal
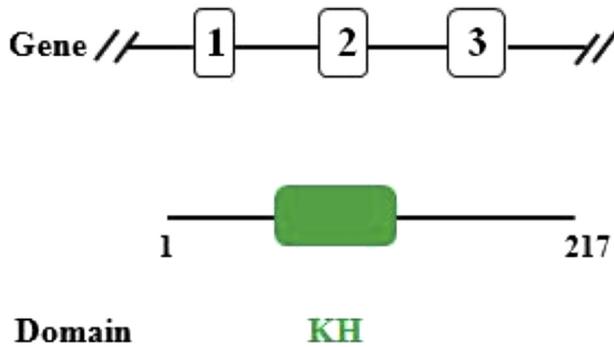
Fig. 3. *KHDC3L* gene has three exons. *KHDC3L* has 217 amino acids and possesses KH domain.

imaging studies were undertaken to fully characterize localization of *NLRP7* and *KHDC3L* proteins in human lymphoblastoid cell lines, peripheral blood mononuclear cells, oocyte and subsequent embryonic stages. In lymphoblastoid cell lines and peripheral blood mononuclear cells these two proteins co-localized in the microtubule organizing center and the Golgi apparatus [19,31]. *NLRP7* intensity was 3 times higher in the cortical region compared to the central region of all oocyte stages from germinal vesicle to metaphase II and fertilized egg. *KHDC3L* was also found to be localized on the cortical area of metaphase II oocytes. Transmission electron microscopy further confirmed the two *NLRP7* and *KHDC3L* proteins to be part of oocyte cytoskeleton with higher concentration in cortical region. Three dimensional confocal z stacks performed on the 2 cell stage embryos revealed absence of *NLRP7* from cell to cell adhesion pole, while it was predominantly localized in the cortical region. Immunofluorescence on dissociated blastomeres from 2 to 8 cell embryos disclosed a quick redistribution of *NLRP7* to the uniform cortical pattern observed in the oocyte stages. *NLRP7* maintained its localization up to morula stage, but was later homogeneously distributed throughout the cytoplasm of both trophectoderm and inner cell mass of the blastocyst. *KHDC3L* on the other hand maintained its localization until morula stage where it began to become nuclear. Nuclear localization of *KHDC3L* was maintained in both trophectoderm and inner cell mass of the blastocyst [11]. This observation suggests that the two genes may have similar or overlapping functions in oocyte and early embryonic development [18].

Epigenetic studies and investigated genes

Epigenetic studies on molar tissues highlighted aberrations of epigenetic marks of imprinted genes. Imprinted genes are small but developmentally important subset of mammalian genes. Their transcription is limited to only one of the two parental alleles and so is sex-dependent. Key elements for the establishment and maintenance of imprinted gene expression are Imprinting control regions (ICR). There are 0.6–10 kb long, CpG-rich sequences, which acquire DNA methylation in the germline in a sex specific manner and so are called differentially methylated regions (DMR). ICR methylation is erased in primordial germ cells (PGCs) and is reestablished in developing gametes and faithfully maintained in zygote and somatic cells during development [32]. Despite the establishment of DNA methylation in male gamete, which occurs in mitotic cells and completes before entering into the meiosis, the establishment of germinal DMR (gDMR) methylation in female occurs in growing oocytes, then becomes arrested in diplotene stage of meiotic prophase I, between the birth and the puberty [33,34].

Studied imprinted genes

DNA methylation aberrations have been found at a wide range of genes, particularly imprinted genes in extracted specimens from molar tissues of patients with *KHDC3L* or *NLRP7* mutations [18], while affected women have normal DNA methylation pattern [35]. It is believed that *KHDC3L* or *NLRP7* mutations by a specific mechanism, which is not well-known so far, affect establishment and/or maintenance of genomic imprinting [36,37]. Several studies have investigated the epigenetic background of Recurrent HYDM and alterations of epigenetic marks particularly methylation as consequences of *NLRP7* mutations. Studied imprinted genes are summarized in Table 1. El-Maarri and colleagues showed hypomethylation for maternally methylated genes *PEG3* and *SNRPN* in products of molar pregnancies [36]. Furthermore, they identified hypermethylation for NESP55 DMR (member of GNAS cluster) and *H19* paternally methylated genes. How paternally imprinted genes, which should emerge unmethylated from oogenesis, are hypermethylated, remains to be clarified [36]. One possibility is that the imprinting marks are not fully erased from Primordial Germ Cells (PGCs) of affected patients and so these patients share an oocyte with hypermethylation on mentioned loci [36]. Methylation of *PEG3*, *SNRPN*, *KCNQ1OT1* and *GNAS exon 1A* (1A) maternally methylated genes was also examined by Kou and colleagues. These loci showed hypomethylation consistent with previous study. *H19* and *NESP55* were unaffected and hypermethylated respectively [38]. *NESP55* secondary imprints are dependent on the correct establishment of the primary maternal imprints at 1A. As, *GNAS exon 1A* (1A) was hypomethylated and *NESP55* became hypermethylated. Methylation for the other components of GNAS cluster, GNAS antisense (AS) DMR and *XLαS* first exon (*XLαS*) was complex [38]. Hayward and colleagues also analyzed methylation of imprinted genes in molar conceptus. They found *ZAC* and *KCNQ1OT1* maternally methylated genes unmethylated [39]. Conversely *SGCE/PEG10*, which is also maternally methylated, preserved methylation imprints [39]. One explanation can be that for SGCE/PEG10, primary imprints are established when *NLRP7* expression is not required or *SGCE/PEG10* imprinting emerges as secondary pattern [39]. Furthermore a recent genome-wide study reveals that in a Recurrent HYDM sample *PEG10* preserves its allelic methylation. *H19* was unaffected and *NESP55* was hypermethylated like previous studies, consistent with the establishment of a paternal epigenotype across the GNAS cluster [39].

One interesting argument is that whether Loss Of Methylation (LOM) due to *NLRP7* mutations makes any difference at expression level. Sanchez-Delgado and colleagues by allelic-specific RT-PCR on the Recurrent HYDM samples showed that, *HYMA1*, *PEG10* and *PEG3* (which are hypomethylated and affected from *NLRP7* mutations), have biallelic expression [40].

Whether *NLRP7* mutations affect non-imprinted genes, is still unclear. It has been shown that upon knockdown of *NLRP7* in human Embryonic Stem Cells (hESCs), there is alteration of methylation for a wide range of CpG islands, but no known imprinted gene was detected among these altered genes [26]. However pyrosequencing analyses of retrotransposable elements (such as LINE-1) reveal normal methylation in Recurrent HYDM samples indicating normal global DNA methylation in these tissues [41]. Studies on 18 identified placenta-specific-maternally methylated DMRs demonstrated that both androgenetic and NLRP7-associated moles were under-methylated on maternal allele [42]. Authors searched for additional loci with abnormal methylation in Recurrent HYDM and 25 more loci associated with placenta-specific-maternally methylated DMRs were found [42]. Beygo and colleagues studied a live-birth case born from a mother with heterozygous *NLRP7* mutation.

Table 1
Summary of studied imprinted genes in recurrent HYDM and observed methylation defect.

Studied DMR	Imprinted allele	Methylation defect	Reference
PEG3	Maternally methylated	Hypomethylation	[41,43]
SNRPN	Maternally methylated	Hypomethylation	[41,43]
H19	Paternally methylated	Hypermethylation, Unaffected	[41,43,44]
NESP55	Paternally methylated	Hypermethylation	[41,43,44]
KCNQ1OT1	Maternally methylated	Hypomethylation	[43,44]
GNAS exon 1A (1A)	Maternally methylated	Hypomethylation	[43]
GNAS antisense	Maternally methylated	Complex	[43]
XLαS first exon (*XLαS*)	Maternally methylated	Complex	[43]
ZAC	Maternally methylated	Hypomethylation	[44]
SGCE/PEG10	Maternally methylated	Unaffected	[44]

Using arrey-based CpG methylation studies in the blood sample of this patient, methylation defects not only for imprinted genes but also for several genes of unknown imprinting status were identified [42].

NLRP7 interactions and suggested mechanism

The main identified mechanism for the role of *NLRP7* maternal-biallelic mutations in the molar pregnancies is the impairment of methylation across maternally imprinted genes during oogenesis. *NLRP7* is a cytoplasmic protein and to make its hypothetical effect on DNA methylation, it should react at chromatin level. Mahadevan and colleagues showed that *NLRP7* interacts with the important chromatin regulator YY1 (Yin Yang 1), the latter in turn can bind to imprinted gDMRs in a methylation-dependent manner to help the coordination of imprinted genes expression. Interestingly, *NLRP7* when overexpressed, can be found in the nucleus and can interact with YY1 in both the nuclear and cytoplasmic compartments [26].

Singer and colleagues in a valuable study introduced ZBTB16 as interaction partner of *NLRP7* [43]. ZBTB16 (also known as PLZF) nuclear protein is a transcriptional repressor and a member of the Krüppel-like zinc finger protein family [44]. Protein mediates its transcriptional silencing through chromatin remodeling via recruitment of DNA histone deacetylases and nuclear co-repressors [45]. Singer and colleagues demonstrated in the mammalian cells *NLRP7* interacts with ZBTB16. Furthermore they showed that when *NLRP7* is overexpressed, the cytoplasmic co-localization of ZBTB16 and *NLRP7* is observed [43]. One possible explanation can be that wild type and mutated *NLRP7* can alter cytokines signaling pathways [46]. Altered cytokines signaling pathways in turn induce redistribution of ZBTB16, which reorganizes chromatin repressive marks. Thus, *NLRP7* affects DNA methylation despite being localized in the cytoplasm. Alternatively, physical interaction of *NLRP7* with ZBTB16 could trap the latter in the cytoplasm and disrupt its nuclear inhibitory function [43].

However the exact mechanism by which *NLRP7* impairs methylation of DNA and the target step of imprinting cycle (establishment of new marks or maintenance of them after puberty and during the wave of demethylation soon after fertilization) remains to be explored. One important example of proteins involved in the maintenance of methylation in gDMRs is DPPA3 (also called PGC7 or STELLA), which plays a crucial role in protecting the maternal genome against DNA demethylation after fertilization. DPPA3 is highly expressed in oocyte and persists in the preimplantation embryo. DPPA3 protects the maternal genome, as well as certain paternally imprinted loci (*H19* and *Rasgraf1*), from loss of methylation. It is confirmed that DPPA3 interacts with H3K9me2-bearing allele (maternal allele) and protects that from TET-dependent demethylation. Conversely the paternal allele, being depleted from histones, becomes actively demethylated soon after fertilization [33,47,48]. In fact DPPA3 and ZFP57 (Zinc finger protein 57 is a member of KRAB-zinc finger protein family) are involved in the methylation maintenance of imprinted genes against TET-dependent demethylation in pronuclei before the first cell division and later. However *NLRP7* role in the proper methylation conservation seems to be antecedent as a genome-wide study shows that epigenetic aberrations arise early in the female germline and paternally methylated DMRs are unaffected.

Another hypothesis for observed hypomethylation of imprinted genes in Recurrent HYDM, concerns histone marks. Establishment of methylation marks is done by *de-novo* DNA methyltransferases DNMT3A and DNMT3B. The DNMT3 group also contains a catalytically inactive member, DNMT3L. DNMT3L has no methyltransferase activity on its own but co-operates with DNMT3A to establish imprinting marks. If in Recurrent HYDM, hypomethylation of imprinted genes arise from any defect of methylation establishment, then the involvement of de-novo methyltransferases is indispensable. DNMT3L is sensitive to Lysine 4 methylations of histone H3. So histone demethylases makes chromatin permissive for DNMT3A/DNMT3L. One notable example of histone demethylases is KDM1B, which demethylates H3K4. Knockout of this protein is associated with defects in establishment of some maternal gDMRs in oocyte. Overall all the above observations, indicate that DNA methylation activity is preceded by chromatin structure and histone marks. One hypothesis is that, mutations of KDM1B or other members of this family, destined to perform these histone modifications, whiche make chromatin ready for DNA methyltransferases, contribute to the methylation aberrations in Recurrent HYDM [40,49,50].

Conclusions

In the present study we have reviewed clinical and genetic bases of Hydatidiform moles with the respect to recurrent type. Genetic studies concerning Recurrent HYDMs are limited, because the rarity of familial cases and the lack of animal model corresponding to the pathology. Moreover, up to now all conducted studies have used molar tissues for genetic and epigenetic analysis and oocyte as the best biological candidate material for this pathology, never is used. However the main explained mechanism for Recurrent HYDM is aberrant methylation of imprinted genes emerged during oogenesis. The establishment of methylation in human oocyte arises between birth and puberty. This long period of time in human makes oocyte susceptible to epigenetic errors. Nevertheless, specification of the precise step of imprinting cycle, which is affected by the pathology and involved mutations, remains complicated due to lack of a direct cellular system.

Since homozygous mutations in *NLRP7* and *KHDC3L* genes have been shown to be the main cause of Recurrent HYDM, DNA testing for these genes in women with at least two complete or partial HMs is recommended. If results show two defective alleles in one or both responsible genes, ovum donation for the patient is advised.

Conflict of interest

Acknowledgment

References

[1] Sebire NJFR, Foskett M, Rees H, Seckl MJ, Newlands ES. Risk of recurrent hydatidiform mole and subsequent pregnancy outcome following complete or partial hydatidiform molar pregnancy. BJOG 2003;110:22–6.

[2] Loukovaara M, Pukkala E, Lehtovirta P, Leminen A. Epidemiology of hydatidiform mole in Finland, 1975 to 2001. Eur J Gynaecol Oncol 2005;26:207–8.

[3] Almasi A, Almassinokiani F, Akbari P. Frequency of molar pregnancies in health care centers of tehran, iran. J Reprod Infertil 2014;15:157–60.

[4] Tham BW, Everard JE, Tidy JA, Drew D, Hancock BW. Gestational trophoblastic disease in the Asian population of Northern England and North Wales. BJOG 2003;110:555–9.

[5] Aghababaii S, Shobeiri F, Hosseinipanah SM. HYDATIDIFORM mole: a statistical survey in West of Iran. J Postgrad Med Inst 2016;30:80–3.

[6] Parazzini F, La Vecchia C, Mangili G, Caminiti C, Negri E, Cecchetti G, et al. Dietary factors and risk of trophoblastic disease. Obstet Gynecol 1988;158:93–9.

[7] Manokhina IHC, Stephenson MD, McFadden DE, Robinson WP. Maternal NLRP7 and C6orf221 variants are not a common risk factor for androgenetic moles, triploidy and recurrent miscarriage. Mol Hum Reprod 2013;19(8):539–44.

[8] Sasaki S. Clinical presentation and management of molar pregnancy. Best Pract Res Clin Obstet Gynaecol 2003;17:885–92.

[9] Benson CB, Genest DR, Bernstein MR, Soto-Wright V, Goldstein DP, Berkowitz RS. Sonographic appearance of first trimester complete hydatidiform moles. Ultrasound Obstet Gynecol 2000;16:188–91.

[10] Zhou Q, Lei XY, Xie Q, Cardoza JD. Sonographic and Doppler imaging in the diagnosis and treatment of gestational trophoblastic disease: a 12-year experience. J Ultrasound Med 2005;24:15–24.

[11] Akoury E, Zhang L, Ao A, Slim R. NLRP7 and KHDC3L, the two maternal-effect proteins responsible for recurrent hydatidiform moles, co-localize to the oocyte cytoskeleton. Hum Reprod 2015;30:159–69.

[12] Williams D, Hodgetts V, Gupta J. Recurrent hydatidiform moles. Eur J Obstet Gynecol Reprod Biol 2010;150:3–7.

[13] EP SRW. NLRP7 and the genetics of hydatidiform moles: recent advances and new challenges. Front Immunol 2013;4:242.

[14] Reddy R, Nguyen NM, Sarrabay G, Rezaei M, Rivas MC, Kavasoglu A, et al. The genomic architecture of NLRP7 is Alu rich and predisposes to disease-associated large deletions. EJHG 2016;24(10). 1516.

[15] Fallahian M. Familial gestational trophoblastic disease. Placenta 2003;24:797–9.

[16] Fan JB, Surti U, Taillon-Miller P, Hsie L, Kennedy GC, Hoffner L, et al. Paternal origins of complete hydatidiform moles proven by whole genome single-nucleotide polymorphism haplotyping. Genomics 2002;79:58–62.

[17] Fallahian MSN, Savage PM, Seckl MJ, Fisher RA. Mutations in NLRP7 and KHDC3L confer a complete hydatidiform mole phenotype on digynic triploid conceptions. Hum Mutat 2013;34(2):301–8.

[18] Nguyen Ngoc Minh Phuong, Slim Rima. Genetics and epigenetics of recurrent hydatidiform moles:basic science and genetic counselling. Curr Obstet Gynecol Rep 2014;3:55–64.

[19] Zhang P, Dixon M, Zucchelli M, Hambiliki F, Levkov L, Hovatta O, et al. Expression analysis of the NLRP gene family suggests a role in human pre-implantation development. PLoS One 2008;3. e2755.

[20] Fisher RA, Hodges MD, Newlands ES. Familial recurrent hydatidiform mole: a review. J Reprod Med 2004;49:595–601.

[21] Van den Veyver IB, Al-Hussaini TK. Biparental hydatidiform moles: a maternal effect mutation affecting imprinting in the offspring. Hum Reprod Update 2006;12:233–42.

[22] Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet 2006;38:300–2.

[23] Messaed C, Akoury E, Djuric U, Zeng J, Saleh M, Gilbert L, et al. NLRP7, a nucleotide oligomerization domain-like receptor protein, is required for normal cytokine secretion and co-localizes with Golgi and the microtubule-organizing center. J Biol Chem 2011;286:43313–23.

[24] Singer H, Biswas A, Zimmer N, Messaed C, Oldenburg J, Slim R, et al. NLRP7 inter-domain interactions: the NACHT-associated domain is the physical mediator for oligomeric assembly. Mol Hum Reprod 2014;20(10):990–1001.

[25] Tschopp J, Martinon F, Burns K. NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol 2003;4:95–104.

[26] Mahadevan S, Wen S, Wan YW, Peng HH, Otta S, Liu Z, et al. NLRP7 affects trophoblast lineage differentiation, binds to overexpressed YY1 and alters CpG methylation. Hum Mol Genet 2014;23:706–16.

[27] Reubinoff BE, Lewin A, Verner M, Safran A, Schenker JG, Abeliovich D. Intra-cytoplasmic sperm injection combined with preimplantation genetic diagnosis for the prevention of recurrent gestational trophoblastic disease. Hum Reprod 1997;12:805–8.

[28] Fisher RA, Lavery SA, Carby A, Abu-Hayyeh S, Swingler R, Sebire NJ, et al. What a difference an egg makes. Lancet 2011;378:1974.

[29] Akoury E, Gupta N, Bagga R, Brown S, Dery C, Kabra M, et al. Live births in women with recurrent hydatidiform mole and two NLRP7 mutations. Reprod Biomed Online 2015;31:120–4.

[30] Parry DA, Logan CV, Hayward BE, Shires M, Landolsi H, Diggle C, et al. Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. Am J Hum Genet 2011;89:451–8.

[31] Reddy R, Akoury E, Nguyen NMP, Abdul-Rahman OA, Dery Cea. Report of four new patients with protein-truncating mutations in C6orf221/KHDC3L and colocalization with NLRP7. Eur J Hum Genet EJHG 2013;21:957–64.

[32] Riso V, Kukreja H, Anvar Z, Verde G, Sparago A, Acurzio B, et al. ZFP57 maintains the parent-of-origin-specific expression of the imprinted genes and differentially affects non-imprinted targets in mouse embryonic stem cells. Nucleic Acids Res 2016;44(17):8165–78.

[33] Kelsey G, Feil R. New insights into establishment and maintenance of DNA methylation imprints in mammals. Philos Trans R Soc Lond B Biol Sci 2013;368:20110336.

[34] Tomizawa S, Nowacka-Woszuk J, Kelsey G. DNA methylation establishment during oocyte growth: mechanisms and significance. Int J Dev Biol 2012;56:867–75.

[35] El-Maarri OSM, Riviere JB, Oldenburg J, Walter J, Rouleau G, Slim R. Patients with familial biparental hydatidiform moles have normal methylation at imprinted genes. Eur J Hum Genet 2005;13:486–90.

[36] El-Maarri O, Seoud M, Coullin P, Herbiniaux U, Oldenburg J, Rouleau G, et al. Maternal alleles acquiring paternal methylation patterns in biparental complete hydatidiform moles. Hum Mol Genet 2003;12:1405–13.

[37] Judson H, Hayward BE, Sheridan E, Bonthron DT. A global disorder of imprinting in the human female germ line. Nature 2002;416:539–42.

[38] Kou YC, Shao L, Peng HH, Rosetta R, del Gaudio D, Wagner AF, et al. A recurrent intragenic genomic duplication, other novel mutations in NLRP7 and imprinting defects in recurrent biparental hydatidiform moles. Mol Hum Reprod 2008;14:33–40.

[39] Hayward BE, De Vos M, Talati N, Abdollahi MR, Taylor GR, Meyer E, et al. Genetic and epigenetic analysis of recurrent hydatidiform mole. Hum Mutat 2009;30:E629–39.

[40] Sanchez-Delgado M, Martin-Trujillo A, Tayama C, Vidal E, Esteller Mea. Absence of maternal methylation in biparental hydatidiform moles from women with NLRP7 maternal-effect mutations reveals widespread placenta-specific imprinting. PLoS Genet 2015;11, e1005644.

[41] Court F, Tayama C, Romanelli V, Martin-Trujillo A, Iglesias-Platas Iea. Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. Genome Res 2014;24:554–69.

[42] Beygo J, Ammerpohl O, Gritzan D, Heitmann M, Rademacher K, Richter J, et al. Deep bisulfite sequencing of aberrantly methylated loci in a patient with multiple methylation defects. PLoS One 2013;8. e76953.

[43] Singer H, Biswas A, Nuesgen N, Oldenburg J, El-Maarri O. NLRP7, involved in hydatidiform molar pregnancy (HYDM1), interacts with the transcriptional repressor ZBTB16. PLoS One 2015;10(6), 0130416.

[44] Suliman BAXD, Williams BR. The promyelocytic leukemia zinc finger protein: two decades of molecular oncology. Front Oncol 2012;2:74.

[45] Barna M, Merghoub T, Costoya JA, Ruggero D, Branford M, Bergia A, et al. Plzf mediates transcriptional repression of HoxD gene expression through chromatin remodeling. Dev Cell 2002;3:499–510.

[46] Doulatov SNF, Rice KL, Howell L, Zelent A, Licht JD. PLZF is a regulator of homeostatic and cytokine-induced myeloid development. Gene Dev 2009;23(17):2076–87.

[47] Kang J, Kalantry S, Rao A. PGC7, H3K9me2 and Tet3: regulators of DNA methylation in zygotes. Cell Res 2013;23:6–9.

[48] Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura Tea. PGC7/Stella protects against DNA demethylation in early embryogenesis. Nat Cell Biol 2007;9:64–71.

[49] Ciccone DN, Su H, Hevi S, Gay Fea. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. Nature 2009;461:415–8.

[50] Zhang Y, Jurkowska R, Soeroes S, Rajavelu A, Dhayalan Aea. Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. Nucleic Acids Res 2010;38:4246–53.