

RESEARCH ARTICLE

Allelic Characterization of IGF2 and H19 Gene Polymorphisms in Molar Tissues

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Abstract

Background: To investigate the characteristics of allelic distribution of IGF2 and H19 gene polymorphisms in molar tissues compared to normal placentas. **Materials and Methods:** Forty-nine specimens of molar tissues as well as 100 control normal placental tissues, delivered on the same days, were collected. Polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) on 2% agarose gel electrophoresis was conducted to determine the allelic distribution. The *ApaI* polymorphism within exon 9 of IGF2 and the *RsaI* polymorphism within exon 5 of H19 were employed to identify the allelic distribution of the IGF2 and H19 genes, respectively. Then the data for these genes in the molar and normal placenta tissues were compared. **Results:** The allelic distribution of IGF2 genes found in molar tissue were 21 (42.9%) aa (undigested), 10 (20.4%) ab (heterozygous) and 18 (36.7%) bb (digested), while in normal placenta tissue the values were 22 (22%) aa, 51 (51%) ab, and 27 (27%) bb. The allelic distribution of H19 in molar tissues was 8 (16.2%) aa (undigested), 8 (16.3%) ab (heterozygous) and 33 (67.4%) bb (digested) and in normal placental tissue was 16 (16%) aa, 36 (36%) ab and 48 (48%) bb in normal placenta tissue. These results were significantly different with P values of 0.001 and 0.037 for the allelic distribution of IGF2 and H19, respectively. **Conclusions:** Molar tissues showed significant differences of allelic distribution of IGF2 and H19 from normal placenta tissues.

Keywords: Molar pregnancy - normal placenta - H19 gene - IGF2 gene - polymerase chain reaction (PCR)

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Introduction

The complete hydatidiform mole (CHM) is one common type of gestational trophoblastic disease (GTD) characterized as a benign condition. Only 20% of CMH develop into a form of malignancy such as an invasive mole or choriocarcinoma (Biscaro et al., 2015). Due to limitations of basic clinical research regarding GTD, the pathogenesis of CHM is still unclear (Steinberg et al., 2014; Kolomietz et al., 2015). The major characteristic of CHM is the absence of a maternal genome, grossly swollen villi and the absence of fetal tissue. Previous studies demonstrated that genomic imprinting during human embryogenesis is essential in the development of CHM (Arima et al., 1997; Li et al., 2002). This genomic imprinting is parental-origin-specific functional differences between the two alleles. This mechanism is important for proper development of both embryonic and extra-embryonic tissues (Hall, 1990).

Recent studies suggested that the potential role of oppositely imprinted H19 and IGF2 genes in GTD (Mutter et al., 1993; Arima et al., 1997; Kim et al., 2003). The main function of IGF2 is to produce a growth factor in humans. Although bi-allelic expression of IGF2 was found in gynecologic tumors including gestational trophoblastic

neoplasm, IGF2 can be bi-allelically expressed in normal development (Cui et al., 1997). In addition to its well-established function in the control of normal placenta development, IGF2 also has an important role in the pathogenesis of choriocarcinoma (Kim et al., 2003). It has been exhibited that IGF2 promoters can affect the progression of some tumors. On the other hand, H19 has a positive effect on tumor suppressor genes (Zhang et al., 1993). The present study aimed to investigate allelic characteristics of IGF2 and H19 genes in molar tissues compared to those with normal placental tissues.

Materials and Methods

This study was approved by the local ethics committee. All participants gave informed consent before participation in this project.

Subjects and Clinical Samples

All cases of molar pregnancy and normal pregnancy were obtained from Mae Sot Hospital, Mae Sareng Hospital and Chiang Mai University Hospital, Thailand during March, 2012 to May, 2013. The specimens of molar tissue were collected by suction and curettage procedure and the tissue was transferred to Department of Pathology,

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Chiang Mai University Hospital for tissue diagnosis by gynecologic pathologists. The normal placentas were obtained after normal deliveries on the same day of the collected molar fresh tissue. The basic clinical data of each specimen was recorded.

Preparation of Genomic DNA

Forty-nine complete moles and 100 normal placental tissues analyzed in this research were randomly sampled for repeated washing by phosphate buffered saline (GibcoBRL®, GibThai Co., Ltd., Chiang Mai, Thailand) combined with 0.1% polyvinyl alcohol (Sigma®, S.M. Chemical Supplies Co., Ltd., Chiang Mai, Thailand) to remove maternal blood. Genomic DNA was extracted from the molar tissues and normal term placentas by using High Pure PCR Template Preparation Kits (Roche Applied Science, Bangkok, Thailand)

Analysis of IGF2 and H19 Expression

Gene polymorphisms of IGF2 and H19 alleles were examined in 49 complete moles using ApaI polymorphism of the IGF2 gene and RsaI polymorphism of the H19 gene. One hundred normal placentas were also analyzed as controls. Polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) on 2% agarose gel electrophoresis with ethidium bromide staining was employed for allelic determination. ApaI polymorphism within exon 9 of IGF2 was used to identify the allelic distribution of the IGF2 gene. The primers were used as follows: sense strand, 5'-CTT GGA CTT TGA GTC AAA TTG-3', and antisense strand, 5'-GGT CGT GCC AAT TAC ATT TCA-3' (Kim, Park et al. 2003). The amplification resulted in a 292 bp fragment whose allele has an ApaI restriction site at 231 bp. Undigested (a) allele showed a 292-bp fragment and digested (b) allele showed 231- and 61-bp fragments.

RsaI polymorphism within the exon 5 of H19 was used to identify the allelic distribution of H19 gene. The sense primer was 5'-TAC AAC CAC TGC ACT ACC TG-3' (located in exon 4), and the antisense primer was 5'-TGG AAT GCT TGA AGG CGT CT-3' (located in exon 5) (Kim, Park et al. 2003). giving rise to a 655-bp gene fragment after PCR amplification with an RsaI restriction site at 485 bp. The undigested (a) allele showed a 575-bp fragment and digested (b) allele showed 405- and 170-bp fragments.

Data of allelic distribution of ApaI polymorphism within the exon 9 of IGF2 and RsaI polymorphism within the exon 5 of H19 of complete moles and normal placenta samples were collected for statistical analysis.

Statistics

Statistical analysis of the data was performed using Chi-square. A P-value of less than 0.05 was considered significant.

Results

Basic Clinical Data

The majority of the 49 specimens of molar pregnancies came from Mae Sot Hospital. The median age was 29.0

years old with an age range of 16-52 years. The two most common ethnicities were Burmese (56.8%) and Karen (20.5%). Almost 80% of the patients presented with abnormal vaginal bleeding. The median of pretreatment B-hCG was 200,000 mIU/ml with a range of 909-200,000 mIU/ml. Regarding the 100 normal pregnancies, the most common ethnicity was Thai (43.6%) followed by Burmese (29.7%), Hmong (15.8%) and Karen (10.9%). The median age was 26.0 with a range of 15 to 39 years old. The median gestational age was 39 weeks with a range of 32 to 42 weeks and the median birth weight was 3,100 grams with a range from 1350 to 4050 grams.

Allelic Discrimination of ApaI Polymorphism within Exon 9 of IGF2

Electrophoresis on 2% agarose gel confirmed PCR amplification of exon 9 of the IGF2 gene from 49 molar and 100 normal placenta tissues. ApaI digestion of the PCR products revealed heterozygosity for IGF2 gene

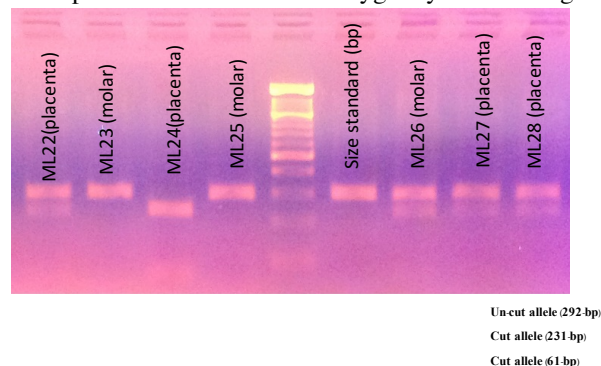


Figure 1. 2% Agarose Gel Electrophoresis Results of ApaI Polymorphism within Exon 9 of IGF2 gene of 3 molar (ML23, ML25 and ML26) and 5 Normal Placenta (ML22, ML24, ML27, ML28 and ML29) Samples. Undigested (a) allele shows a 292-bp fragment and digested (b) allele shows 231- and 61-bp fragments. ML23, ML25 and ML26 samples reveal homozygous un-cut (aa) allele; ML22, ML27, ML28 and ML29 reveal heterozygous (ab) allele; ML24 reveals homozygous cut (bb) allele

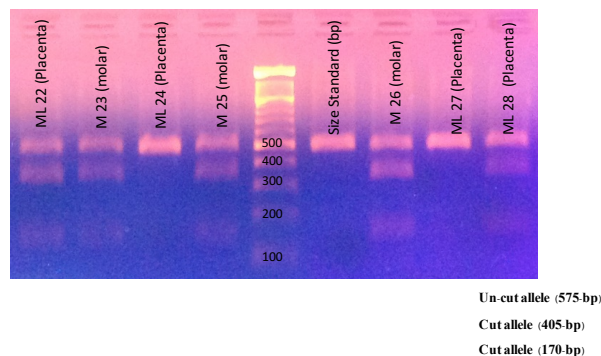


Figure 2. 2% Agarose Gel Electrophoresis Results of RsaI Polymorphism within exon 5 of H19 gene of 3 molar (ML23, ML25 and ML26) and 5 Normal Placenta (ML22, ML24, ML27, ML28 and ML29) Samples. Undigested (a) allele gives rise to a 575-bp fragment and digested (b) allele gives rise to a 405- and 170-bp fragments. ML24, ML26 and ML28 samples reveal homozygous un-cut (aa) allele; ML22, ML23, ML25, ML27 and ML29 reveal heterozygous (ab) allele

Table 1. Allelic Distribution of ApaI Polymorphism within Exon 9 of IGF2 Gene and RsaI Polymorphism within Exon 5 of H19 Gene in Molar Tissues and Normal Placentas

Gene	Molar Tissues (total = 49)	Normal Placenta (total = 100)	P Value
IGF2 Alleles			0.001
aa (un-digested)	21 (42.9%)	22 (22.0%)	
ab (heterozygous)	10 (20.4%)	51 (51.0%)	
bb (digested)	18 (36.7%)	27 (27.0%)	
H19			0.037
aa (un-digested)	8 (16.3%)	16 (16.0%)	
ab (heterozygous)	8 (16.3%)	36 (36.0%)	
bb (digested)	33 (67.4%)	48 (48.0%)	

as shown in Figure 1. Allelic distribution of IGF2 gene comprised of 21 (42.9%) aa (undigested), 10 (20.4%) ab (heterozygous) and 18 (36.7%) bb (digested) in molar tissues, and 22 (22%), 51 (51%), and 27 (27%) in normal placenta, respectively. It is evident that molar tissues showed a higher incidence of aa ApaI allele of IGF2 gene than that of normal placenta tissues with statistical significance (Chi-square = 13.51, P-value = 0.001) as noted in Table 1.

Allelic Discrimination of RsaI Polymorphism within Exon 5 of H19

2% Agarose gel electrophoresis following PCR amplification and RsaI digestion exhibited heterozygosity of the H19 gene. The analysis was performed on 49 molar and 100 normal placenta tissues. Allelic distribution of H19 contained 8 (16.2%) aa (undigested), 8 (16.3%) ab (heterozygous) and 33 (67.4%) bb (digested) in molar tissues; and 16 (16%) aa, 36 (36%) ab and 48 (48%) bb in normal placenta tissues, respectively as showed in Figure 2. The findings suggested that molar tissues show a higher incidence of bb RsaI allele of H19 gene than that of normal placenta tissues with statistical significance (Chi-square = 6.58, P-value = 0.037).

Discussion

This study demonstrated that the allelic distribution of IGF2 and H19 genes in molar tissues and normal placenta tissues was different. Although normal placenta tissue showed variables in allelic characteristics, molar tissues exhibited dominate aa ApaI allele of IGF2 gene and bb RsaI allele of H19 gene. LOI of IGF2 (bb allele) in this study was 36.7% in molar tissues that agreed with a recent study suggesting that the loss of imprinting of the IGF2 gene may play a role in the development and maintenance of malignancy in GTD (Kim et al., 2003) Like proto-oncogene, the full mutation gene will develop neoplasia. In fact, approximately 20% of complete moles will turn to neoplasia (Biscaro et al., 2015).

A previous study suggested that the relaxation of H19 imprinting was expressed in the androgenic tissue of a complete hydatidiform mole (Ariel et al., 1994; Kim et al., 2003). The results of this study indicated that the loss of imprinting H19 genes (bb allele) was mostly found in molar tissues (67.4%). All normal placentas should

maintain normal allelic characteristics of the imprinting genes including IGF2 and H19 genes (Kim et al., 2003). Another study in contrast to our study showed that the LOI of the IGF2 gene was 27% and the LOI of the H19 gene was 48%. The mechanism to explain these results were still unclear, while variable allelic characteristics of IGF2 and H19 genes in individual normal placenta tissues was observed.

Compared to previous studies in this field, the number of samples in this study were considerably high. Due to the large sample size, this study presented various imprinting genes of normal placentas and complete moles. The high number of the investigated samples can be considered as a strength of this research. Further study may follow the patients who had molar pregnancies with variable allelic characteristics of imprinting IGF2 and H19 genes to observe the potential development into gestational trophoblastic neoplasia in the future.

In conclusion, molar tissue showed a higher incidence of IGF2 (aa allele) and H19 (bb allele) than normal term placenta tissue with statistical significance. This study demonstrated that allelic distribution of IGF2 and H19 might be associated associated with gestational trophoblastic disease.

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