Prognostic effect of ABO blood group on women with androgenetic complete hydatidiform moles

（雄核発生全胞状奇胎の予後に対するABO 血液型の影響）

千葉大学大学院医学薬学府
先端医学薬学専攻
（主任：生水真紀夫教授）
佐藤　明日香

Prognostic effect of ABO blood group on women with androgenetic complete hydatidiform moles

Asuka Sato

Department of Reproductive Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan
1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.

Contents

Acknowledgments

I would like to express my gratitude to Professor Makio Shozu for providing me this study opportunity as a Ph.D. student and for insightful suggestion. I especially would like to express my appreciation to my supervisor, Dr. Hirokazu Usui for his elaborated guidance, considerable encouragement and invaluable discussion. I am grateful to Dr Zijun Pan for technical assistance. I also would like to thank Prof. Tohru Iseki and Mrs. Hiroko Hasegawa for the assistance and valuable discussion of blood transfusion testing. I am grateful to the staff of the Department of Obstetrics and Gynecology at Chiba University Hospital for treating the patients and for their assistance with sample collection.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding information

This work was partially supported by JSPS KAKENHI grant numbers JP17K16831

Abstract

Introduction: Complete hydatidiform moles (CHMs) can develop into gestational trophoblastic neoplasia (GTN). Because CHMs are allografts to the patients in terms of an androgenetic origin, some immunological reactions may be involved in the development of GTN from CHMs. However, no study has analyzed the relationship between ABO types of moles and GTN occurrence, especially from the viewpoint of immune compatibility. This study aimed to evaluate the effect of ABO blood group on the prognosis of androgenetic CHMs.

Material and methods: A total of 129 patients who were diagnosed as having CHMs based on multiplex short tandem repeat polymorphism analysis between January 2007 and February 2016 were included. The ABO blood types of the patients were determined serologically and those of molar tissues were determined by single nucleotide polymorphisms in the ABO gene using a high-resolution melting assay with real-time polymerase chain reaction. The incidence of GTN was compared based on patients' ABO blood types and the ABO compatibility between patients and their molar tissues.

Results: The overall incidence of GTN was 17.1% (22/129). GTN occurred in 10.8% (4/37), 14.8% (8/54), 22.2% (6/27), and 36.4% (4/11) of type O, A, B, and AB patients, respectively (P = 0.202, Fisher's exact test). Type AB patients tended to develop GTN compared with other blood type patients (P = 0.093, Fisher's exact test). In ABO type of CHMs, GTN occurrence was not significantly different as 16.4% (10/64), 16.0% (8/50), and 22.2% (4/18) of type O, A, and B CHMs, respectively (P = 0.854, Fisher's exact test). The ABO types of patients and molar tissues were immunologically compatible in 94 cases and incompatible in 35 cases. GTN occurred in 18 (19.1%) of the compatible cases and 4 (11.4%) of the incompatible cases; the occurrence was not significantly different (P = 0.223).

Conclusions: Patients with type AB tended to develop GTN compared with those with types A, B, and O. However, ABO compatibility between patients and molar tissues had no relationship with GTN occurrence. Another GTN developing mechanism would exist rather than immunological rejection based on ABO type of androgenetic CHMs.

Keywords

ABO blood group system, blood group incompatibility, gestational trophoblastic neoplasia, hydatidiform mole, immunological rejection, organ transplantation

Abbreviations

CHM, complete hydatidiform mole; GTN, gestational trophoblastic neoplasia; HM, hydatidiform mole; HRM, high-resolution melting; PHM, partial hydatidiform mole; STR, short tandem repeat

Introduction

Hydatidiform mole (HM) is an abnormal pregnancy characterized by swollen chorionic villi and proliferative trophoblast cells. It has the potential risk for developing gestational trophoblastic neoplasia (GTN). The two categories of HMs according to their cytogenetic origin are complete HMs (CHMs) as androgenetic diploid and partial HMs (PHMs) as diandric monogynic triploid.[1] The incidences of GTN from CHMs and PHMs are 15-20% and 1-2%, respectively.[1, 2] Thus, androgenetic CHMs are major source of GTN, but the developing mechanism of GTN has not been revealed. Characteristically, both CHMs and GTN from CHMs are complete allografts for patients because they are derived from conceptus tissues without maternal chromosomes.[1, 2] Development of GTN from CHMs may be attributable to histocompatibility between patients and their molar tissues.

The ABO blood type is a major human alloantigen system that is composed of A and B antigens expressed on red blood cells, and anti-A and anti-B antibodies against those antigens in serum. In addition to their expression on red blood cells, ABO antigens are expressed in other human cells and tissues.[3] Recently, numerous studies about the relationship between ABO blood types and various diseases have been reported.[4-8] A few studies indicated the effect of ABO blood types against the outcomes of pregnancy such as preeclampsia,[9] but the effects were discordance among the studies.

The association between ABO blood types and gestational trophoblastic diseases has been investigated for the last several decades. In the 1970s, the combination of patient's and her husband's ABO blood types was applied on the risk factor to predict the sensitivity of single agent chemotherapy for GTN.[10-12] Even in Japan, a regional cohort study of 4,547 HM patients showed that a patient with blood type AB is associated with GTN occurrence.[13] These reports suggested that immunological reaction by ABO antigen and antibody may play a role in eliminating neoplastic trophoblasts on the process of developing GTN from HMs. The patients with blood type AB might not reject HMs because they do not have anti-A nor anti-B antibody. However, no study has analyzed the relationship between ABO blood types and GTN from the viewpoint of immune compatibility because of the difficulty of determination of ABO types of HMs.

In this study, we aimed to evaluate the effect of ABO blood types on the risk of GTN from CHMs in a retrospective observational cohort setting. We focused on androgenetic CHMs only

but not on PHMs genetically diagnosed using consecutive cases to assess the genuine effect of ABO blood types on the incidence of GTN. We hypothesized that ABO immune compatibility correlated with GTN occurrence. To evaluate them, we determined the ABO types of CHM using single nucleotide polymorphism (SNP) genotyping. The incidence of GTN was then compared based on ABO compatibility between patients and their molar tissues.

Material and methods

Patient recruitment and clinical management

Patients were referred to our hospital because of suspected molar pregnancy between January 2007 and February 2016. After transvaginal ultrasonographic examination and preoperative evaluation including blood tests of human chorionic gonadotropin (hCG) and blood types, evacuation of uterine content was performed in Chiba University Hospital. Intrauterine tissues were submitted to the pathological department for routine histopathological evaluation. After the evacuations, the patients were followed up by monitoring serum hCG levels weekly, biweekly, and monthly before they reach to 50 mIU/mL, 10 mIU/mL, and cutoff level, respectively. GTN was diagnosed using the FIGO 2000 criteria.[14] In cases of GTN, we further evaluated the patient using imaging studies such as transvaginal sonography, chest X-ray, and chest and abdominal computed tomography. We classified them as low- or high-risk according to the FIGO 2000 risk score.[14]

Molecular genetic diagnosis

For patients who consented to join the molecular genetic diagnostic study, we performed short tandem repeat (STR) polymorphism analysis using the patient's blood and the villous tissues as reported previously.[15-17] Briefly, DNA extraction of the blood and tissues was performed using QIAamp DNA Mini Kit and QIAamp Blood DNA Mini Kit (QIAGEN, Tokyo, Japan), respectively, according to the manufacturer's instructions. STR analysis was conducted using a PowerPlex 16HS Kit (Promega, Madison, WI, USA), as previously described.[17] Diagnostic algorithm was previously reported in detail. If one or more villous loci did not show any maternal alleles, the case was classified as an androgenetic CHM; otherwise, it was classified as a biparental conceptus. In a biparental conceptus, based on the existence of three allelic loci or the lack thereof, the concepti were classified as diandric monogynic PHM or biparental abortion, respectively.

Subject selection and clinical data collection

Patients with androgenetic CHM classified by STR analysis were enrolled into this study (Figure 1). PHM cases and biparental abortion cases were excluded. Hysterectomy cases were included. Patients with complete hydatidiform moles coexisting with a live fetus were also included. Clinical and laboratory data including patient's age, pre-evacuated serum hCG, and ABO blood type were collected from medical records.

Determination of ABO histo-blood type of patients

ABO histo-blood type of the patients was determined using standard erythrocyte antiserum agglutination methods at the clinical laboratory department of Chiba University Hospital.

Determination of ABO genotype of patients and molar tissues

ABO genotype was determined using DNA samples extracted from patients' blood and molar tissues for molecular diagnosis. The A and B antigens are converted from H antigen by the glycosyltransferase activity encoded by the ABO gene.[3] The ABO gene with type A allele encodes N-acetylgalactosaminyltransferase, which converts H antigen into A antigen, whereas that with type B allele encodes galactosyltransferase, which converts H antigen into B antigen. Type O allele makes the frame shift and encodes incomplete peptide chain without glycosyltransferase activity.[3] The ABO gene is located on chromosome 9, which genotype is known to be defined by SNPs (Figure 2A).[3]

To determine the ABO genotype, we used real-time polymerase chain reaction (PCR) and high-resolution melting (HRM) assay, which detect the difference in melting pattern of each amplicon. We targeted two SNP loci: rs8176719 in exon 6 and rs8176749 in exon 7 (Figure 2A). Primers were designed to amplify 67-bp products for rs8176719 (forward 5′-TCCATGTGCAGTAGGAAGGA-3′ and reverse 5′-GTGCCCTCCCAGACAATG-3′) and 70-bp products for rs8176749 (forward 5′-ACGAGAGCCACCTGAACAAG-3′ and reverse 5′-CACAAGTACTCGGGGGAGAG-3′). Real-time PCR was performed using the LightCycler® Nano Instrument (Roche Diagnostics, Tokyo, Japan). The PCR mixtures consisted of genomic DNA (3 ng), 1 × LightCycler® 480 High Resolution Melting Master (Roche Diagnostics, Tokyo, Japan), 2.5 mM $MgCl_2$, and 200 nM of each forward and reverse primer in a total 10 μl volume. The thermocycling conditions used for both primer pairs were as follows: 95 °C for 10 min; 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 25 s, extension at 72 °C for 15 s, then hold at 95 °C for 60 seconds and 40 °C for 60 seconds, and finished by HRM procedure

from 60 °C to 99 °C at 0.050 °C/s. The absence of artifacts and nonspecific PCR products was confirmed by agarose gel electrophoresis. HRM analysis were performed using LightCycler NanoSoftware 1.1 (Roche Diagnostics, Tokyo, Japan). Genotyping experiments with HRM analysis was repeated independently twice.

The representative results of HRM genotyping were confirmed by the Sanger method using BigDye Terminator Kit, version 1.1 (Applied Biosystems, Foster City, CA) and ABI prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Algorithm of the estimation of blood types from the HRM data

HRM analyses of rs8176719 and rs8176749 loci make the SNP patterns as G/G, G/del, del/del and G/G, G/A, A/A, respectively (Figure 2B). The algorithm of estimated genotypes and blood types is shown in Table 1.

Determination of ABO compatibility

Incompatible cases were defined as the cases that patients had the antibody against the antigen encoded by the ABO gene of molar tissues. According to that definition, type A patients are incompatible with type BB, BO and AB CHMs. Type B patients are incompatible with type AA, AO and AB CHMs. Type O patients are incompatible with all types except for type OO of CHMs, and type AB patients were compatible with all genotypes of CHMs.

Statistical analysis

Data are presented as median (interquartile range), means ± standard deviation, or number and percentage. Statistical analysis was performed using JMP® Pro 13 (SAS Institute Inc., Cary, NC, USA). Fisher's exact test was used for categorical variables. Statistical analysis for multiple groups was performed using one-way ANOVA, Kruskal-Wallis test, and Fisher’s exact test depending on the valuable type. Cochran-Armitage trend test was used to investigate the GTN incidence for the antibody score, which we defined as the number of anti-A and/or anti-B retention antibodies; we assigned the antibody score as 2, 1, 1, and 0 for blood types O, A, B, and AB, respectively. The association between ABO compatibility and GTN occurrence was analyzed using Fisher’s exact test.

Ethical approval

The study protocols were approved by Institutional Review Board (Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University, No. 674 and No. 882) and the research was performed after obtaining written, informed consent from each participant.

Results

Enrolled subjects and demographic characteristics

In total, 206 patients were referred to Chiba University Hospital for suspected molar pregnancy and underwent evacuation, except one patient who was treated with primary hysterectomy. We compared the patient's blood and their villous tissue with multiplex STR analysis. One hundred and thirty-three patients were diagnosed with androgenetic CHM by STR analysis. Among them, four patients were excluded from this study because the DNA samples extracted from the molar tissues were insufficient to determine ABO blood type. Finally, 129 patients were enrolled into this retrospective observational cohort study (Figure 1).

Of the patients, 37 (28.7%), 54 (41.8%), 27 (20.9%), and 11 (8.5%) were serologically classified as types O, A, B, and AB, respectively (Table 2). The ABO blood type distribution of the subjects was approximately equal to the general Japanese population.[18] The patient's age, gravidity, parity, gestational weeks at termination of pregnancy, and pre-evacuated serum hCG levels were not different among each group (Table 2). One hundred and eleven (86.0%) patients were classified to have homozygous CHMs, whereas the other patients were classified to have heterozygous CHMs. The distribution did not differ by the patients' blood types.

Relationship between patients' ABO blood types and GTN incidence

The GTN incidence was 17.1% in total. All GTN patients were classified as low-risk GTN using FIGO 2000 risk score.[14] The incidences were varied from 10.8% (type O) to 36.4% (type AB) (P = 0.202, Fisher's exact test for 2 × 4 matrix) (Table 3). Type AB patients tended to develop GTN compared with the other blood type patients (P = 0.093, Fisher's exact test for 2 × 2 matrix). This result suggested that patients who have neither anti-A nor anti-B antibodies had higher potential to develop GTN. Thus, we further analyzed the trend test by assigning the antibody score as described in the Material and methods section. The GTN incidence was significantly higher in patients with lower antibody score (P = 0.036, Cochran-Armitage trend test) (Table 3).

Validation of ABO blood type determination of HRM assay

We performed HRM analysis for the two SNP loci (rs8176719 and rs8176749) (Figure 2A). Representative HRM curves are shown in Figure 2B. Three genotypes as G/G, del/del, G/del on rs8176719, and G/G, A/A, A/G on rs8176749 could be separated clearly. According to the determination algorithm of blood type (Table 1), all patients' blood types were determined using

the HRM assay. Compared with the patients' histo-blood type to the determined blood types, all cases were in agreement (Table 4). Thus, HRM assay could be used to determine ABO types of the molar tissues.

ABO blood type of molar tissues

The ABO genotype of molar tissues was classified as OO, AA, AO, BB, BO, and AB in 61, 45, 5, 17, 1 and 0 patients, respectively (Table 5). Most CHMs were homozygous; thus, heterozygous genotypes, such as AO, BO, and AB, were rare. This distribution was compatible with the one estimated using the Japanese allele frequency (Table 5).[18] The GTN incidence was not significantly different by ABO types of molar tissues ($P = 0.854$, Fisher's exact test) (Table 5).

Relationship between ABO compatibility and GTN incidence

Compatibility of ABO blood type was determined according to the combination of the patient's and molar tissue's ABO blood type as shown in Table 6. The ABO blood types of patients and molar tissues were compatible in 94 cases and incompatible in 35 cases. GTN occurred in 18 (19.1%) of the compatible cases and 4 (11.4%) of the incompatible cases, but the occurrence was not significantly different ($P = 0.223$) (Table 7).

Discussion

In this study, we revealed that type AB patients were associated with GTN occurrence, whereas it could not be explained by ABO immune compatibility. Using the serological method of blood type determination, direct comparison of immune compatibility between the patients and the mole was impossible because the mole's ABO type could not be determined. To the best of our knowledge, we introduced a genotyping method to determine ABO types of CHM. We hypothesized that the ABO immune compatibility between the patients and their moles should determine the development of GTN.

In 1971, Bagshawe et al. described the relationship between the ABO blood type and trophoblastic neoplasia, which is now called GTN.[10, 11] The relationship between the mating patterns of ABO blood type and the prognosis of GTN was evaluated. Based on the data, the combination of ABO blood type of patients and their husbands had been adopted in the WHO risk score.[12] Later, ABO blood type was discounted in the new version of the risk score of GTN (FIGO 2000 risk score)[14, 19] because their significance had not been confirmed.[20, 21] The older version of WHO risk score used the ABO blood types to classify low- or high-risk GTN. Our

study discussed about the risk of GTN development from CHMs because the hypothesis that immunological rejection might affect in eliminating trophoblasts after evacuation of HM would be attractive.

Patients with AB blood type tended to have a higher risk of GTN, but it was not statistically significant. The result was consistent with the previous cohort study in Aichi prefecture in Japan; the study included both types of HM (CHM and PHM).[13] Patients with blood type AB do not have anti-A antibody nor anti-B antibody. Thus, they might not reject the ABO-incompatible allografts in addition to the compatible allografts. In this background, we raised the ABO immune compatible hypothesis for developing GTN. However, the compatibility of ABO types between the patients and their molar tissues did not correlate with the GTN occurrence. Thus, there seemed to be another relationship between patients' ABO blood types and GTN occurrence. From the viewpoint of the immunological hypothesis, the individual difference of ABO antibody titers may affect the development of GTN. Anti-A and anti-B antibodies include IgG, IgM, and IgA, and their titers are influenced mainly by environmental factors.[22] Lower antibody titers may make it difficult to eliminate circulating trophoblasts after CHM evacuation, resulting in GTN occurrence. Another hypothesis was that the expression level of ABO antigens on the trophoblast surface differed among each CHM and affected the immunological rejection. ABO antigens have been reported not to express in the placenta, but no data are available about the expression of ABO antigen on the circulating trophoblast surface. This point of view requires future evaluation.

Except for the immunological hypothesis, another possible explanation for the relationship between ABO blood type and GTN occurrence is that the activity of coagulation activity or cell adhesion molecules may be related to ABO blood type. Recently, the relationship between ABO blood type and the disease or the various pathological conditions, such as thrombotic vascular diseases,[7] coronary artery disease,[5] cancer development,[4] and mortality of severe trauma,[8] has been reported. AB blood type could be related to coagulation tendency as thrombotic features.[7] If the clots with molar trophoblast cells were the starting materials of metastasis, the higher risk of AB blood type might be acceptable. Moreover, genetic variation in ABO gene has been reported to correlate with circulating levels of proinflammatory cytokines and cell adhesion molecules, such as tumor necrosis factor alpha,[23] soluble intracellular adhesion molecule 1,[24] soluble E-selectin, and P-selectin.[25] These factors play an important role in carcinogenesis and tumor

metastasis.

Evaluation of GTN incidence is not easy because the basal population of CHM and PHM among molar pregnancies might be different among studies. The discrimination between CHM and PHM could be difficult in particular cases.[16] The incidences of GTN are quite different according to the types of molar pregnancy — 15-20% from CHMs and 1-2% from PHMs.[1, 2] In this study, we used consecutive androgenetic CHM cases, which were most reliable in diagnostic accuracy.[1, 15] The uniform selection of patients in this study was an advantage. The limitation of this study was the small sample size; thus, the tendency discussed above was not conclusive. GTN is a rare tumor. A nationwide study is warranted to resolve the question.

Conclusion

Type AB patients tended to develop GTN. However, ABO compatibility between patients and their molar tissues did not show significant relationship with GTN occurrence. Higher GTN incidence in AB blood type patients could not be explained by the immunological rejection mechanism based on ABO type of androgenetic CHMs.

References

1. Hui P, Buza N, Murphy KM, Ronnett BM. Hydatidiform Moles: Genetic Basis and Precision Diagnosis. Annu Rev Pathol. 2017;12:449-485.
2. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. Lancet. 2010;376:717-729.
3. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990;345:229-233.
4. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, Arslan AA, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet. 2009;41:986-990.
5. Chen Z, Yang SH, Xu H, Li JJ. ABO blood group system and the coronary artery disease: an updated systematic review and meta-analysis. Scientific reports. 2016;6:23250.
6. Franchini M, Liumbruno GM, Lippi G. The prognostic value of ABO blood group in cancer patients. Blood Transfus. 2016;14:434-440.
7. Vasan SK, Rostgaard K, Majeed A, Ullum H, Titlestad KE, Pedersen OB, et al. ABO Blood Group and Risk of Thromboembolic and Arterial Disease: A Study of 1.5 Million Blood Donors. Circulation. 2016;133:1449-1457; discussion 1457.
8. Takayama W, Endo A, Koguchi H, Sugimoto M, Murata K, Otomo Y. The impact of blood type O on mortality of severe trauma patients: a retrospective observational study. Crit Care. 2018;22:100.
9. Franchini M, Mengoli C, Lippi G. Relationship between ABO blood group and pregnancy complications: a systematic literature analysis. Blood Transfus. 2016;14:441-448.
10. Bagshawe KD, Rawlins G, Pike MC, Lawler SD. ABO blood-groups in trophoblastic neoplasia. Lancet. 1971;1:553-556.
11. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. Cancer. 1976;38:1373-1385.
12. World Health Organization Scientific Group. Gestational trophoblastic disease. Technical report series 692. Geneva, Switzerland: World Health Organization, 1983.
13. Hara T, Kaseki S, Tomoda Y, Nishikawa Y, Ishizuka T, Ishizuka N. Determinants of risk for developing invasive mole and choriocarcinoma following hydatidiform mole. Asia Oceania J Obstet Gynaecol. 1986;12:241-250.

14. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. Int J Gynaecol Obstet. 2002;77:285-287.
15. Usui H, Qu J, Sato A, Pan Z, Mitsuhashi A, Matsui H, et al. Gestational Trophoblastic Neoplasia From Genetically Confirmed Hydatidiform Moles: Prospective Observational Cohort Study. Int J Gynecol Cancer. 2018;28:1772-1780.
16. Usui H, Kiyokawa T, Qu J, Nishikimi K, Tate S, Mitsuhashi A, et al. Comparison Between Pathological Diagnosis and Cytogenetic Diagnosis by Short Tandem Repeat Polymorphism Analysis of Suspected Molar Pregnancies. J Reprod Med. 2016;61:219-223.
17. Baasanjav B, Usui H, Kihara M, Kaku H, Nakada E, Tate S, et al. The risk of post-molar gestational trophoblastic neoplasia is higher in heterozygous than in homozygous complete hydatidiform moles. Hum Reprod. 2010;25:1183-1191.
18. Maeda K, Nakamura S, Murakami C, Irie W, Watanabe T, Sasaki C, et al. ABO genotyping by TaqMan assay and allele frequencies in a Japanese population. Leg Med (Tokyo). 2013;15:57-60.
19. Kohorn EI, Goldstein DP, Hancock BW, Kim SJ, Lurain JR, Newlands E, et al. Workshop Report: Combining the staging system of the International Federation of Gynecology and Obstetrics with the scoring system of the World Heath Organization for Trophoblastic Neoplasia. Report of the Working Committee of the International Society for the Study of Trophoblastic Disease and the International Gynecologic Cancer Society. Int J Gynecol Cancer. 2000;10:84-88.
20. Ngan HY, Lopes AD, Lauder IJ, Martin BH, Wong LC, Ma HK. An evaluation of the prognostic factors in metastatic gestational trophoblastic disease. Int J Gynecol Cancer. 1994;4:36-42.
21. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. Obstet Gynecol. 1994;84:969-973.
22. de Franca ND, Poli MC, Ramos PG, Borsoi CS, Colella R. Titers of ABO antibodies in group O blood donors. Rev Bras Hematol Hemoter. 2011;33:259-262.
23. Melzer D, Perry JR, Hernandez D, Corsi AM, Stevens K, Rafferty I, et al. A genome-wide association study identifies protein quantitative trait loci (pQTLs). PLoS Genet. 2008;4:e1000072.

24. Pare G, Chasman DI, Kellogg M, Zee RY, Rifai N, Badola S, et al. Novel association of ABO histo-blood group antigen with soluble ICAM-1: results of a genome-wide association study of 6,578 women. PLoS Genet. 2008;4:e1000118.
25. Liumbruno GM, Franchini M. Hemostasis, cancer, and ABO blood group: the most recent evidence of association. J Thromb Thrombolysis. 2014;38:160-166.

Figure 1

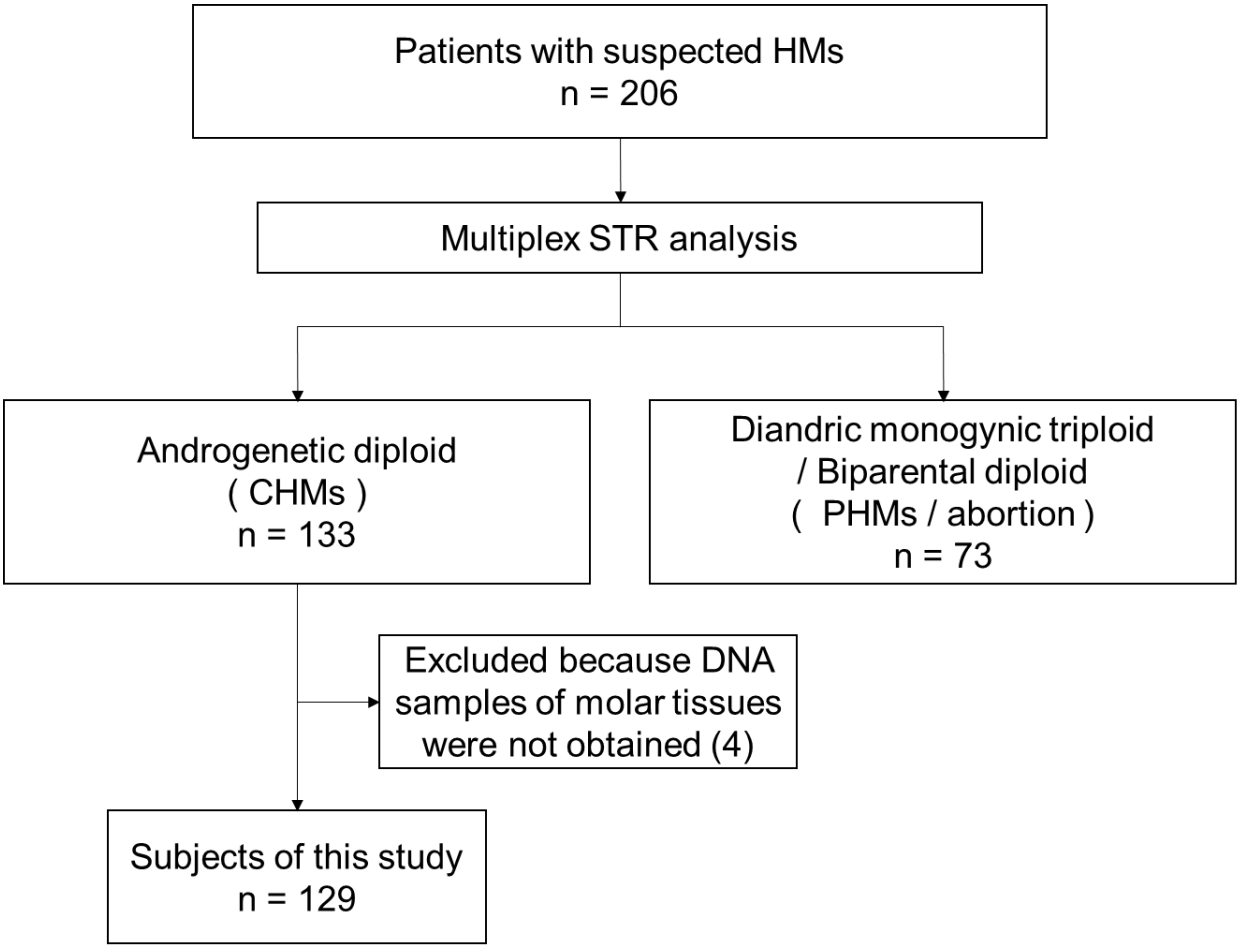
Patients with suspected HMs
n = 206
Multiplex STR analysis
Androgenetic diploid
(CHMs)
n = 133
Diandric monogynic triploid
/ Biparental diploid
(PHMs / abortion)
n = 73
Excluded because DNA
samples of molar tissues
were not obtained (4)
Subjects of this study
n = 129

Figure 2

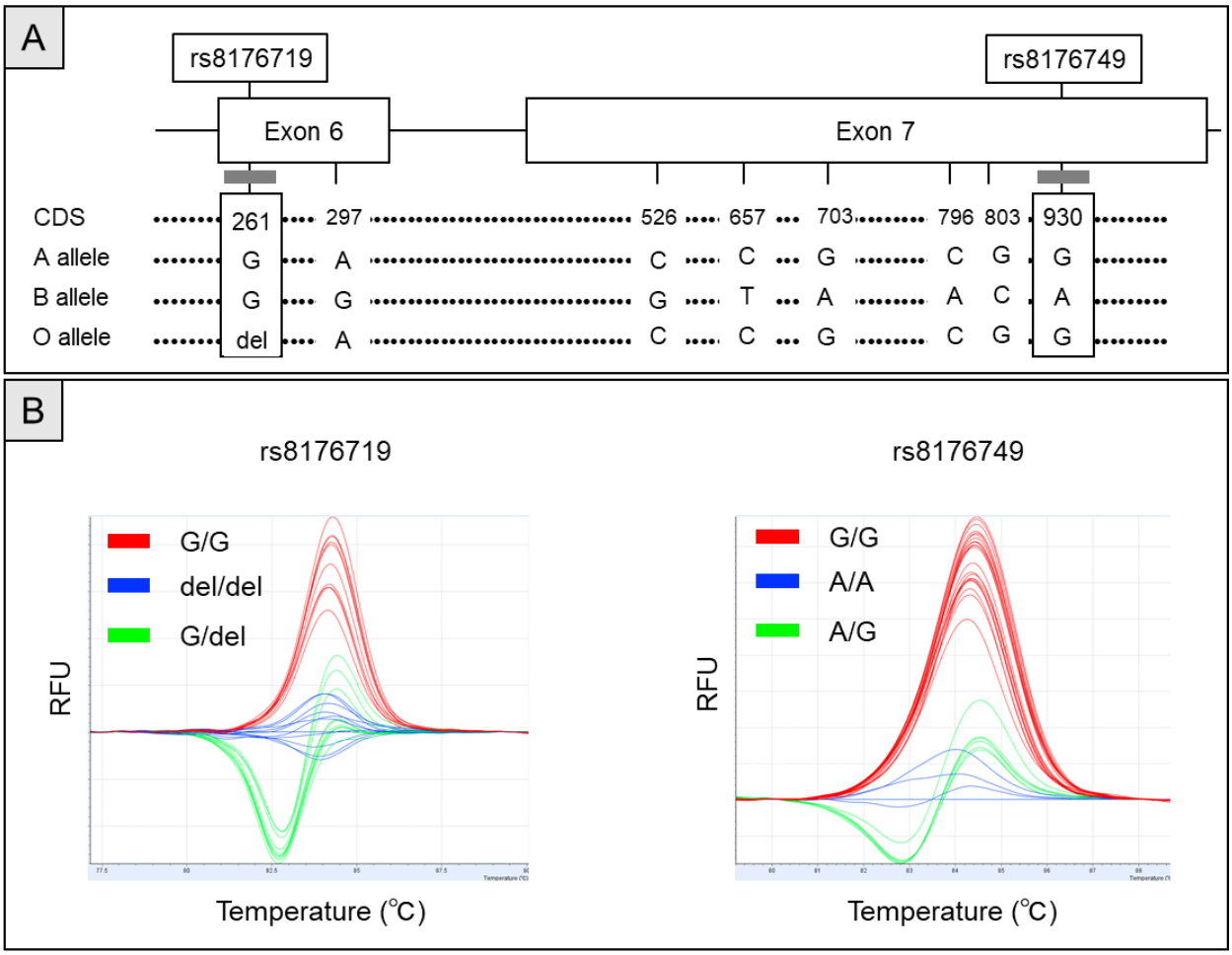
A
rs8176719
rs8176749
Exon 6
Exon 7
CDS
A allele
B allele
O allele
261
297
526
657
703
796
803
930
G
A
C
C
G
C
G
G
G
G
G
T
A
A
C
A
del
A
C
C
G
C
G
G
B
rs8176719
rs8176749
G/G
del/del
G/del
G/G
A/A
A/G
RFU
RFU
Temperature (°C)
Temperature (°C)

Figure legends

Figure 1 Flow diagram for selection of study subjects.
CHM, complete hydatidiform mole; HM, hydatidiform mole; PHM, partial hydatidiform mole; STR, short tandem repeat

Figure 2 ABO genes, single nucleotide polymorphisms, and discrimination using the high-resolution melting assay.
A: Schematic presentation of exon 6 and exon 7 of ABO gene. A, B, and O alleles have grouped single nucleotide polymorphisms, which could determine the genotypes using the combination of the two loci. B: High-resolution melting (HRM) assay for rs8176719 discriminated the three genotypes as G/G, del/del, and G/del. C: HRM assay for rs8176749 discriminated the three genotypes as G/G, A/A, and G/A.

Table 1

Algorithm of the estimation of blood types from the SNP genotyping data

rs8176719		rs8176749		Determined genotype	Histo-blood type
SNP	Possible genotype	SNP	Possible genotype		
G/G	AA AB BB	G/G	AA AO OO	AA	A
G/G	AA AB BB	G/A	AB BO	AB	AB
G/G	AA AB BB	A/A	BB	BB	B
G/del	AO BO	G/G	AA AO OO	AO	A
G/del	AO BO	G/A	AB BO	BO	B
G/del	AO BO	A/A	BB	*	
del/del	OO	G/G	AA AO OO	OO	O
del/del	OO	G/A	AB BO	*	
del/del	OO	A/A	BB	*	

*These combinations between the two loci should not be assigned theoretically.

Table 2

Baseline characteristics in each blood group of the patients

	Total N = 129	Type O N = 37	Type A N = 54	Type B N = 27	Type AB N = 11	*P* value
Frequency (%)		28.7	41.8	20.9	8.5	
Reported Japanese frequency [a] (%)		29.4	38.3	22.3	10.0	
Characteristics						
Age, years (±SD)	32.2 (±7.7)	31.3 (±8.0)	31.4 (±7.9)	33.2 (±6.3)	36.0 (±7.6)	0.23[b]
Gravidity, median (IQR)	2 (1–7)	2 (1–5)	2.5 (1–7)	3 (1–6)	2 (1–6)	0.08[c]
Parity, median (IQR)	1 (0–5)	0 (0–3)	1 (0–5)	1 (0–4)	0 (0–3)	0.12[c]
TOP, weeks, median (IQR)	9.6 (7–24)	9.9 (7–24)	9.9 (7–17)	9.1 (7–19)	10.3 (8–16)	0.25[c]
Pre-evacuated hCG, mIU/mL median (IQR)	155805 (14332–1892762)	148321 (14332–1120000)	166261 (19857–1680784)	142491 (33479–1155847)	183793 (69360–1892762)	0.39[c]
Cytogenetic classification						
Homozygous CHM (%)	111 (86.0)	31 (83.8)	49 (90.7)	21 (77.8)	10 (83.8)	0.41[d]
Heterozygous CHM (%)	18 (14.0)	6 (16.2)	5 (9.3)	6 (22.2)	1 (16.2)	

[a] Maeda K, Nakamura S, Murakami C, et al. ABO genotyping by TaqMan assay and allele frequencies in a Japanese population. *Leg Med (Tokyo)*. 2013;15(2):57-60.

[b] One-way ANOVA

[c] Kruskal-Wallis test

[d] Fisher's exact test

Table 3

Patients' ABO blood types and GTN occurrence

	Total	Type O	Type A	Type B	Type AB	*P* value
n	129	37	54	27	11	
GTN (%)	22 (17.1%)	4 (10.8%)	8 (14.8%)	6 (22.2%)	4 (36.4%)	0.202[a]
		18 (15.3%)			4 (36.4%)	0.093[a]
Antibody score*		2	1	1	0	
Anti-A antibody		+	−	+	−	
Anti-B antibody		+	+	−	−	
Incidence of GTN		10.8%	17.3% (14/81)		36.4%	0.036[b]

* Antibody score is defined as the number of A and B antibodies.

[a] Fisher's exact test

[b] Cochran-Armitage trend test

Table 4

Validation of the determined ABO blood type by genotyping of HRM assay compared with the patient's histo-blood type

Patient's genotype	Determined ABO blood type	Patient's histo-blood type			
		O	A	B	AB
OO	Type O	37	0	0	0
AA	Type A	0	14	0	0
AO		0	40	0	0
BB	Type B	0	0	3	0
BO		0	0	24	0
AB	Type AB	0	0	0	11

Table 5

Distribution of molar ABO genotypes and GTN occurrence

Mole genotype	n	(%)	Estimated frequency (%)[a]	GTN (%)[b]		Histo-blood type
OO	61	47.2	52.9	10 (16.4)	10 (16.4)	O
AA	45	34.8	27.1	8 (17.8)	8 (16.0)	A
AO	5	3.9	1.5	0		
BB	17	13.2	16.9	3 (17.7)	4 (22.2)	B
BO	1	0.8	1.0	1		
AB	0	0	0.5	0	0	AB

[a]Estimated frequency was calculated based on the reported Japanese ABO allele frequency (A : B : O = 28.1 : 17.7 : 54.2)*, assuming that homozygous CHMs accounted 90% of all CHMs.

[b]Incidence of GTN was not significantly different according to ABO histo-blood type of moles (P = 0.854, Fisher's exact test).

*Maeda K, Nakamura S, Murakami C, et al. ABO genotyping by TaqMan assay and allele frequencies in a Japanese population. *Leg Med (Tokyo)*. 2013;15(2):57-60.

Table 6

Combination of patient's and mole's ABO blood type

Mole genotype	Patients' blood type[a]				Total (n)
	O	A	B	AB	
OO	14	28	14	5	61
AA	**16**	19	**6**	4	45
AO	**2**	2	**1**	0	5
BB	**5**	**5**	5	2	17
BO	**0**	**0**	1	0	1
AB	**0**	**0**	**0**	0	0

[a]Bold numbers indicate the ABO incompatible combination between the anti A/B antibody of patients and the ABO antigen on the moles.

Table 7

Relationship between ABO incompatibility and development of gestational trophoblastic neoplasia

	Total	GTN (%)	
Compatible	94	18 (19.1%)	*P* = 0.223[a]
Incompatible	35	4 (11.4%)	

[a]Fisher's exact test