

# The synonymous 903C>G mutation in the alpha 1,4-galactosyltransferase gene in a Chinese woman with habitual abortion

## A case report

Xiaoying Lv, Bachelor, Degree<sup>a</sup>, Yongquan Chen, MD<sup>b</sup>, Yuanyuan Luo, MD<sup>c</sup>, Lingbo Li, Doctor, Degree<sup>d,\*</sup>, Houzhao Wang, MD<sup>a,\*</sup>

### Abstract

**Rationale:** Habitual abortion is caused by complex and diverse factors, such as genetic factors, immune factors, endocrine factors, viruses, bacterial infections, and so on. Allogeneic antibodies, generated due to blood-group incompatibilities between a female and her fetus, are sometimes important for habitual abortion.

**Patient concerns:** A 26-year-old woman had undergone abortions 3 times in July 2015 (17 weeks pregnant), March 2017 (15 weeks of gestation) and February 2018 (16 weeks pregnant) before she came to the Reproductive Medicine Center of our hospital for prenatal examinations without pregnancy.

**Diagnoses:** Unexplained habitual abortion.

**Interventions:** A series of serological tests and nucleotide sequence of 1,4-galactosyltransferase (A4GALT) gene were performed.

**Outcomes:** The patient was the rare p phenotype in P1P<sup>k</sup> blood system and the patient's habitual abortion was caused by anti-PP1P<sup>k</sup> antibody which was generated naturally in persons with p phenotype. There was a mutation (903C>G, CCC>CCG) in the 3rd exon of A4GALT gene, which is likely a significant contributor to p phenotype.

**Lessons:** This is the first case of habitual abortion caused by p phenotype due to independent 903C>G homozygous mutation with no similar record reported before, which indicates that it is a new class of mutation that leads to p phenotype.

**Abbreviations:** A4GALT = 1,4-galactosyltransferase, BAGMD = Blood Group Antigen Gene Mutation Database, HDFN = hemolytic disease of the fetus and newborn.

**Keywords:** A4GALT gene, anti-PP1P<sup>k</sup> antibody, habitual abortion, p phenotype, P1P<sup>k</sup> blood system

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Written informed consent was obtained from the patient and her sister for publication of this report and any accompanying images.

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<sup>a</sup> Department of Clinical Laboratory, No.174 Hospital of Chinese People's Liberation Army/Chengong Hospital Affiliated to Xiamen University/174 Clinical College of Anhui Medical University, <sup>b</sup> Department of Clinical Laboratory, Xiamen Humanity Hospital, <sup>c</sup> Department of Blood Transfusion, Xiamen Maternal and Child Health Hospital, Xiamen, Fujian, <sup>d</sup> Changchun Bioxun Biotechnology Limited Liability Company, Changchun, Jilin, China.

\* Correspondence: Houzhao Wang, Master degree, Department of Clinical Laboratory, No.174 Hospital of Chinese People's Liberation Army / Chengong Hospital Affiliated to Xiamen University/174 Clinical College of Anhui Medical University, 94 Wenyuan Road, Xiamen City, Fujian Province, 361003, China (e-mail: wanghouzhao@126.com); Lingbo Li, Doctor degree, Changchun Bioxun Biotechnology Limited Liability Company, 285 Xinpu Road, Changchun, Jilin Province, 130102, China (e-mail: lilingbo@bioxun.com).

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## 1. Introduction

Habitual abortion, known as recurrent miscarriage, is clinically defined by 2 or more consecutive pregnancy failures or stillbirths<sup>[1]</sup>, which leads to great physical and psychological damage to the patients. The causes of habitual abortion are complex and diverse, and recent studies have shown that it is associated with genetic factors, immune factors, endocrine factors, viruses, bacterial infections, and so on<sup>[2,3]</sup>. In regard to immune factors, the allogeneic antibodies generated due to the blood-group incompatibilities of female individuals and their fetus are sometimes important reasons for habitual abortion<sup>[4]</sup>. For example, when female individuals in RhD (–) blood type are pregnant with fetus in RhD (+) blood type, they will be faced with a high abortion rate. In some other blood type systems, such as the P1P<sup>k</sup> blood system, irregular antibodies are generated naturally, which can also cause the occurrence of habitual abortion in pregnant women<sup>[5,6,7]</sup>.

Recently, we encountered a patient who likely suffered habitual abortion due to anti-PP1P<sup>k</sup> (anti-Tja) antibody generation. After serological and molecular biology tests on the patient and her family members, we confirmed that the patient and her sister possessed p phenotype, and the habitual abortion that occurred in the patient was caused by the anti-PP1P<sup>k</sup> antibody. The p phenotype is a rare phenotype in P1P<sup>k</sup> blood system, individuals with p phenotype lack all of the antigens of the P1P<sup>k</sup>

**Table 1**  
The ABO, Rh blood group identification results of the patient and her spouse.

|         | Forward typing |        |        |        |                     | Reverse typing  |                 | Self cell |
|---------|----------------|--------|--------|--------|---------------------|-----------------|-----------------|-----------|
|         | Anti-A         | Anti-B | Anti-D | Anti-H | A <sub>1</sub> cell | B cell          | O cell          |           |
| Patient | 0              | 4+     | 4+     | 2+     | 4+                  | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 0         |
| Sister  | 0              | 4+     | 4+     | 2+     | 4+ <sup>W</sup>     | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 0         |
| Spouse  | 0              | 0      | 4+     | 4+     | 4+                  | 4+              | 0               | 0         |

"S" represents slightly stronger, "W" represents slightly weaker, "0" represents no agglutination, "1~4+" represents agglutination intensity gradually increases. The ABO blood group of the patient and her sister showed abnormalities with B type in forward typing and O type in reverse typing, the patient's mother was a normal B type, the patient's spouse was a normal O type, the RhD of all the members were positive.

blood system and possess anti-PP1P<sup>k</sup> antibody in their serum, which is a naturally occurring antibody with both IgG and IgM isoforms. The anti-PP1P<sup>k</sup> antibody can agglutinate all the other types of phenotypic erythrocytes except for the p phenotype in P1P<sup>k</sup> blood system, which can lead to habitual abortion in early pregnancy. For the generating mechanism, the p phenotype is controlled by the alpha 1,4-galactosyltransferase (A4GALT) gene which is located on chromosome 22 (22q13.2) and consists of 4 exons. Specifically, exon 3 contains all the gene coding sequences and translation initiation sites, which encode 360 amino acids. Any mutation of exon 3 that produces an amino acid change may cause A4GALT inactivation, leading to antigen loss of P1, P<sup>k</sup>, P, or/and LKE, and therefore, forming the p phenotype<sup>[8]</sup>.

Considering the specificity of p and anti-PP1P<sup>k</sup> antibody, as well as the serious impact on pregnant women, timely detection, correct identification and diagnosis of p phenotype are of great significance for maternal pregnancy and effective treatment. This study will provide a reference for the future study of causes of p phenotype and habitual abortion, which can enrich the immunological factors of habitual abortion.

## 2. Case presentation

A 26-year-old woman (the proband), who had undergone abortions 3 times in July 2015 (17 weeks pregnant), March 2017 (15 weeks of gestation) and February 2018 (16 weeks pregnant), came to the Reproductive Medicine Center of our hospital for prenatal examinations without pregnancy. The chief complaints of the patient indicated that she had an abortion history with no history of blood transfusion, trauma, genetic or other infectious disease. The physical examination showed no obvious abnormalities, and the results of routine tests (blood routine tests, urine routine tests, liver, kidney function, etc) were all normal. No significant abnormalities were observed in the routine examination for the patient's spouse.

However, The ABO blood group tests showed abnormalities: the forward and reverse typing results of the patient were inconsistent with B type in forward typing and O type in reverse typing. Followed by tracking the blood type of her family members, we found that the patient's sister reported the same abnormalities in ABO blood group while the patient's spouse had a normal O type. All members were positive in RhD tests. The specific response patterns are listed in Table 1.

Considering that there may be allogeneic antibodies present in the serum of the patient and her sister, we conducted the serum irregular antibody screening test, and the results for both the patient and her sister were consistent: the I, II and III spectral cells were all positive in the conditions of saline medium (1000g immediately centrifuged) and indirect anti-human globulin, and the cells showed a complete hemolysis after incubation at 37°C

for 45 minutes. The specific response patterns are listed in Table 2.

Since the antibody types in the serum of the patient and her sister could not be determined by the results of irregular antibody screening, we conducted the irregular antibody identification test, and the results were consistent between the patient and her sister: all 10 kinds of human spectral erythrocytes (Shanghai blood biological medicine limited liability company, China, batch number 20160304) were positive in the conditions of saline medium (1000g immediately centrifuged) and indirect anti-human globulin, and the cells showed a complete hemolysis after incubation at 37°C for 45 minutes. To further explore the antibody properties, we first incubated the positive serum in 56°C for 30 minutes to inactivate the complements and then used 2-mercaptoethanol to inactivate the IgM antibodies. After these treatments, we used the prepared serum to repeat the irregular antibody identification test, and the results showed that all 10 types of human spectral erythrocytes were negative in the conditions of saline medium (1000g immediately centrifuged), while positive in the conditions of indirect anti-human globulin, and the cells showed no hemolysis after incubation at 37°C for 45 minutes. The specific response patterns are listed in Table 3.

From the irregular antibody identification results above, we assessed that there were multi-reactive, high-frequency antibodies present in the serum of the patient and her sister. These antibodies consisted of both IgM and IgG and possessed complementary activity. However, we still could not determine the type of the antibody. Compared with the response pattern table of antibody identification spectrum cells, we speculated that the antibodies could be 1 or more variants of anti-PP1P<sup>k</sup> antibody, anti-Kp<sup>b</sup> antibody, anti-Lu<sup>b</sup> antibody. So, then we selected the corresponding reagents to perform serological antibody identification tests. The results showed that reactions between the erythrocytes of the patient and her sister with human anti-PP1P<sup>k</sup> antibody

**Table 2**  
The irregular antibody screening results of the patient and her sister.

|                              | serum + reagent red blood cells |                 |                 |           |
|------------------------------|---------------------------------|-----------------|-----------------|-----------|
|                              | I cell                          | II cell         | III cell        | Self cell |
| Immediately centrifuged      | 3                               | 3+              | 3+ <sup>S</sup> | 0         |
| Indirect anti-human globulin | 3+ <sup>S</sup>                 | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 0         |
| 37°C 45min                   | H                               | H               | H               | 0         |

"S" represents slightly stronger, "W" represents slightly weaker, "H" represents completely hemolytic status, "0" represents no agglutination, "1~4+" represents agglutination intensity gradually increases. The irregular antibody screening test for the patient and her sister showed that the results were all positive when the serum reacted with the I, II, and III spectral cells in the conditions of saline medium (1000g immediately centrifuged) and indirect anti-human globulin, the cells showed a completely hemolytic status after being placed in 37°C for 45 minutes.

**Table 3****The irregular antibody identification test results of the patient and her sister.**

|                              | Serum + 10 kinds of human spectral erythrocytes |                 |                 |                 |                 |                 |                 |                 |                 |                 |
|------------------------------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                              | 1   | 2               | 3               | 4               | 5               | 6               | 7               | 8               | 9               | 10              |
| Immediately centrifuged      | 3+  | 3+              | 3+              | 3+              | 3+              | 3+              | 3+              | 3+              | 3+              | 3+              |
| Indirect anti-human globulin | 3+ <sup>S</sup>                                 | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> | 3+ <sup>S</sup> |
| 37°C 45min                   | H   | H               | H               | H               | H               | H               | H               | H               | H               | H               |
| 2-Me treated (saline medium) | 0   | 0               | 0               | 0               | 0               | 0               | 0               | 0               | 0               | 0               |
| 2-Me treated (IAT)           | 2+  | 2+              | 2+              | 2+              | 2+              | 2+              | 2+              | 2+              | 2+              | 2+              |

"S" represents slightly stronger, "H" represents complete hemolytic status, "0" represents no agglutination, "1~4+" represents agglutination intensity gradually increases, 2-Me represents 2-mercaptoethanol. The irregular antibody identification test showed that there were multi-reactive high-frequency antibodies in the serum of the patient and her sister, the antibodies contained both IgM and IgG and possessed complement activity: the IgM antibody could lead to hemolysis in vivo while the IgG antibody could not.

**Table 4****The P1Pk blood group identification results of the patient and her sister.**

| Reaction conditions            | Erythrocytes from the patient and her sister  | Normal control O cells                        | Serum from the patient and her sister | Serum from the patient and her sister |
|--------------------------------|---|---|---------------------------------------|---------------------------------------|
|                                | +   | +   | +                                     | +                                     |
|                                | Human anti-PP1P <sup>k</sup> antibody reagent | Human anti-PP1P <sup>k</sup> antibody reagent | Human p-type erythrocytes             | Normal control O cells                |
| Centrifuge at room temperature | 0   | 3+  | 0                                     | 2+ <sup>S</sup>                       |

"S" represents slightly stronger, "0" represents no agglutination, "1~4+" represents agglutination intensity gradually increases. The human anti-PP1P<sup>k</sup> antibody reagent and human p-type erythrocytes were provided by Shanghai Blood Center, the normal control O cells was the red blood cells from ABO reverse typing reagent. The P1P<sup>k</sup> blood group identification results showed that the patient and her sister were both p phenotype in P1P<sup>k</sup> blood system with anti-PP1P<sup>k</sup> antibodies in their serum.

reagent were negative, and the reactions between the serum of the patient and her sister with human p-type erythrocytes were negative, while the normal control O cells were positive. Therefore, we initially determined that the patient and her sister possessed the rare p phenotype in P1P<sup>k</sup> blood system with anti-PP1P<sup>k</sup> antibodies in their serum; the specific response patterns are listed in Table 4. At the same time, the patient's spouse was identified as type P1.

As shown above, there was anti-PP1P<sup>k</sup> antibody in the serum of the patient and her sister. To further understand the antibody properties, we detected the antibody titer, which indicated an IgM antibody titer of 1:32 and IgG antibody titer of 1:16. The specific response patterns are listed in Table 5.

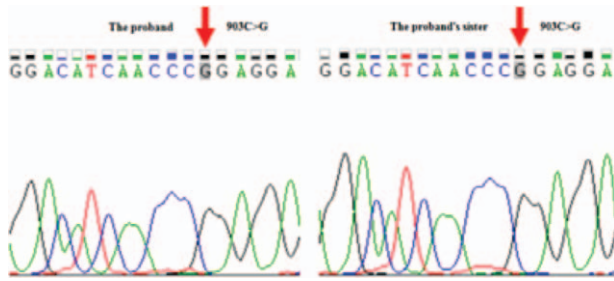
According to all of the reports about p phenotype in Blood Group Antigen Gene Mutation Database (BAGMD), the p phenotype was 1 form of recessive inheritance and all cases of p phenotype were associated with the mutations of A4GALT gene. Therefore, we sequenced the A4GALT genes of the patient and her sister to further explore the generating mechanism of this type of habitual abortion. The results of polymerase chain

reaction-sequence specific primer (PCR-SSP) tests showed that there were allelic mutations in the samples of the sisters. According to positive and reverse sequencing results, 903C>G synonymous mutations, appearing in the sequence of GGA-CATCAACCCG(C>G)GAGGA at the position 903, were found in the patient and her sister, which differed from the reference sequence (GenBank AJ245581) for the normal population. Moreover, the peak of the sequencing map showed that the allelic mutations of the 2 sisters in the 903 bp site of exon 3 within the A4GALT gene (903C>G, CCC>CCG) were homozygous. By searching for mutations of p in BAGMD, the 903C>G mutation had never appeared separately, so we continued to sequence and analyze other exons of the A4GALT genes of the 2 sisters to find whether there was(were) any other mutation(s), while no additional mutations were found. To summarize, the independent occurrence of the 903C>G homozygous mutation caused the p phenotype, and this is the first case of p phenotype formed due to the independent occurrence of 903C>G homozygous mutation. The sequencing results are shown in Figure 1 and Figure 2.

**Table 5****The detect of anti-PP1P<sup>k</sup> antibody titer in the serum of the patient and her sister.**

|  | 2                    | 4  | 8   | 16  | 32  | 64 | 128 | 256 | 512 |
|--|----------------------|----|-----|-----|-----|----|-----|-----|-----|
| Saline medium                            | The patient          | 2+ | 2+  | 1+  | 1+w | ±  | 0   | 0   | 0   |
|  | The patient's sister | 2+ | 2+  | 1+  | 1+  | ±  | 0   | 0   | 0   |
| 2-Me treated                             | The patient          | ±  | 0   | 0   | 0   | 0  | 0   | 0   | 0   |
|  | The patient's sister | ±  | 0   | 0   | 0   | 0  | 0   | 0   | 0   |
| Indirect anti-human globulin tests (IAT) | The patient          | 1+ | 1+  | 1+w | ±   | 0  | 0   | 0   | 0   |
|  | The patient's sister | 1+ | 1+w | 1+w | ±   | 0  | 0   | 0   | 0   |

"0" represents no agglutination, "W" represents slightly weaker, "1~4+" represents agglutination intensity gradually increases. The results of the antibody titer tests for the anti-PP1P<sup>k</sup> antibodies in the sisters showed that the titer of IgM antibody was 1:32 and the titer of IgG antibody was 1:16.



**Figure 1.** The positive sequencing results of the sisters. The positive and reverse sequencing results showed that 903 C>G (CCC>CCG) synonymous mutations (homozygous mutations) were found within exon 3 of the A4GALT gene in both of the 2 sisters.

### 3. Discussion

Many red blood cell system antibodies have important clinical significance, such as roles in hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs)<sup>[9]</sup>. A retrospective study of clinically significant antibodies appearing in the Chinese population suggested that irregular antibodies that caused severe HDFN involved more than 10 blood group antibodies from nearly 10 blood systems<sup>[10]</sup>. In addition to leading to HDFN, some irregular antibodies that are produced in blood systems, such as the Rh blood system, MNS blood system, and P1P<sup>k</sup> blood system, can even cause habitual abortion in pregnant women<sup>[5,6,7]</sup>.

The P blood system was discovered by Landsteiner and Levine when they developed a study of hemolytic disease in the newborn in 1927. At present, the P blood system is renamed P1P<sup>k</sup> blood system by the International Society of Blood Transfusion (ISBT), including P1, P<sup>k</sup>, and NOR antigens which are synthesized by the enzyme encoded by the A4GALT gene<sup>[11]</sup>. The rare “naked” phenotype p is formed when the erythrocyte lacks of all the antigens of P1P<sup>k</sup> blood system<sup>[12]</sup>. Anti-PP1P<sup>k</sup> antibody, which can agglutinate all of the other phenotypic erythrocytes except for the p phenotype, is generated naturally in the serum of all p phenotype individuals, and studies have shown that anti-PP1P<sup>k</sup> antibodies can lead to habitual abortion during early pregnancy in women<sup>[13,14]</sup>. The anti-PP1P<sup>k</sup> antibody contains both IgG and IgM isoforms which can rapidly remove donor erythrocytes by acting on the structures of P, P1, and P<sup>k</sup> antigen carbohydrates,

which is an important cause of abortion in p phenotype women<sup>[13]</sup>. In this study, according to comprehensive detection, the patient and her sister were proved to possess the rare p phenotype in the P1P<sup>k</sup> blood system. The accidental antibodies in the serum of the sisters were frequently anti-PP1P<sup>k</sup> antibodies, and the habitual abortion that occurred in the patient was predominantly caused by the anti-PP1P<sup>k</sup> antibodies.

As a kind of autosomal stealth inheritance blood group<sup>[6]</sup>, only homozygous mutation showed the rare p phenotype, while the heterozygotes mutation did not. Furthermore, p phenotype often appeared in the same generation<sup>[15]</sup>. Studies<sup>[12]</sup> have shown that the Cluster of Differentiation 77 (CD77) synthetic enzyme gene-A4GALT gene determines the p phenotype and many kinds of mutations appearing in the A4GALT gene can result in the generation of p phenotype. In this case, we found that both the proband and her sister were p phenotype in P1P<sup>k</sup> blood system, and the sequencing results showed that the allelic mutations of the patient and her sister were homozygous in the 903 site of exon 3 within the A4GALT gene (903C>G,CCC>CCG). The 903C>G mutation is a kind of single nucleotide polymorphism that belongs to the synonymous mutation of allele (amino acid proline at position 301 does not change). This means that the replacement of some single nucleotides produces a new codon, but does not alter the encoded amino acid, which is a form of a silent mutation<sup>[16]</sup>. The synonymous mutation of 903C>G has never appeared alone among all the p phenotype reported in the BAGMD. However, in this study, only the 903C>G mutation was found in the p phenotype and the p phenotype formed only when the 903C>G mutation was homozygous. This is the first case of p phenotype formed due to the independent occurrence of the 903C>G homozygous mutation. To summarize, through the serum and molecular biology research of these 2 cases, we confirmed that the patient and her sister were both rare p phenotype. It was the synonymous mutation of 903C>G in the A4GALT gene that causes the p phenotype in P1P<sup>k</sup> blood system and it was the in vivo anti-PP1P<sup>k</sup> antibody of the patient that caused habitual abortion.

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### Author contributions

**Data curation:** Xiaoying Lv, Chen Yongquan, Yuanyuan Luo.

**Methodology:** Xiaoying Lv, Chen Yongquan, Yuanyuan Luo, Lingbo Li.

**Validation:** Xiaoying Lv, Chen Yongquan, Yuanyuan Luo.

**Writing - Original Draft:** Chen Yongquan.

**Writing - Review & Editing:** Chen Yongquan.

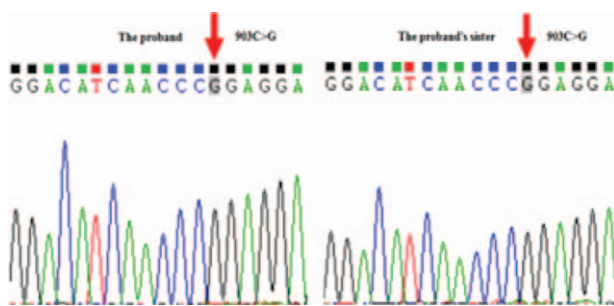
**Formal analysis:** Houzhao Wang, Lingbo Li.

**Project administration:** Houzhao Wang, Lingbo Li.

**Resources:** Houzhao Wang, Lingbo Li.

**Supervision:** Houzhao Wang, Lingbo Li.

Chen Yongquan orcid: 0000-0003-4085-4329.



**Figure 2.** The reverse sequencing results of the sisters. The positive and reverse sequencing results showed that 903 C>G (CCC>CCG) synonymous mutations (homozygous mutations) were found within exon 3 of the A4GALT gene in both of the 2 sisters.

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