行政院國家科學委員會專題研究計畫 成果報告

P 血型系統的分子遺傳學研究(3/3)
研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2320-B-002-053-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學生化科學研究所

計 畫 主 持 人：余榮熾

計畫參與人員：碩士級-專任助理：謝粧衣、王詩欣、王厚仁、侯旻如
博士後研究：易蘭葛文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 08 月 28 日

P 血型系統的分子遺傳學研究

報告內容

前言、

The P Blood Group System

The P blood group system was discovered by Landsteiner and Levine in 1927 (1), and was expanded by Sanger in 1955 (2). The International Society of Blood Transfusion (ISBT) Terminology Working Party has assigned the system number 003 and the symbol P1 to the P system. The P system contains only one antigen, P1. There are three additional antigens, P, P^k, and LKE, closely related to the P1 antigen, but they are not included in the P system because P and LKE antigens are not controlled by genes at the same locus as that governing P1, and the position of P^k in the P system is still unclear (reviewed in Ref. 3) although the gene responsible for the P^k antigen expression has been cloned recently (4).

Biochemistry of the P1, P, P^k, and LKE Antigens

The structures and the biochemical steps involved in the syntheses of P1, P, and P^k antigens (as shown in Fig. 1 in the next page) were elucidated through the efforts by Morgan and Watkins (5,6) and Naiki and Marcus (7). The P1 antigen is characterized by the carbohydrate structure Galα1-4Galα1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer, and synthesis of the P1 epitope is determined by the activity of α-1,4-galactosyltransferase acting on the paragloboside precursor (Galα1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer). The paragloboside is also the precursor for the type 2 H, i and I antigens. The type 2 H structure can be further modified to produce A and B antigens.

The structures of the P, P^k, and LKE antigens belong to the globoside series (Fig. 1). The P^k antigenic structure (Galα1-4Galβ1-4Glcβ1-1Cer) (CTH or CD77) is produced after the action of α-1,4-galactosyltransferase on the lactosylceramide (CDH) precursor. Then the P antigenic structure (GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer) (globoside) can be synthesized as the P^k structure serving as a precursor through the action of the β-1,3-*N*-acetylgalactosaminyltransferase activity. The LKE antigen (NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer) is produced after the

addition of two specific carbohydrate residues on the P structure.

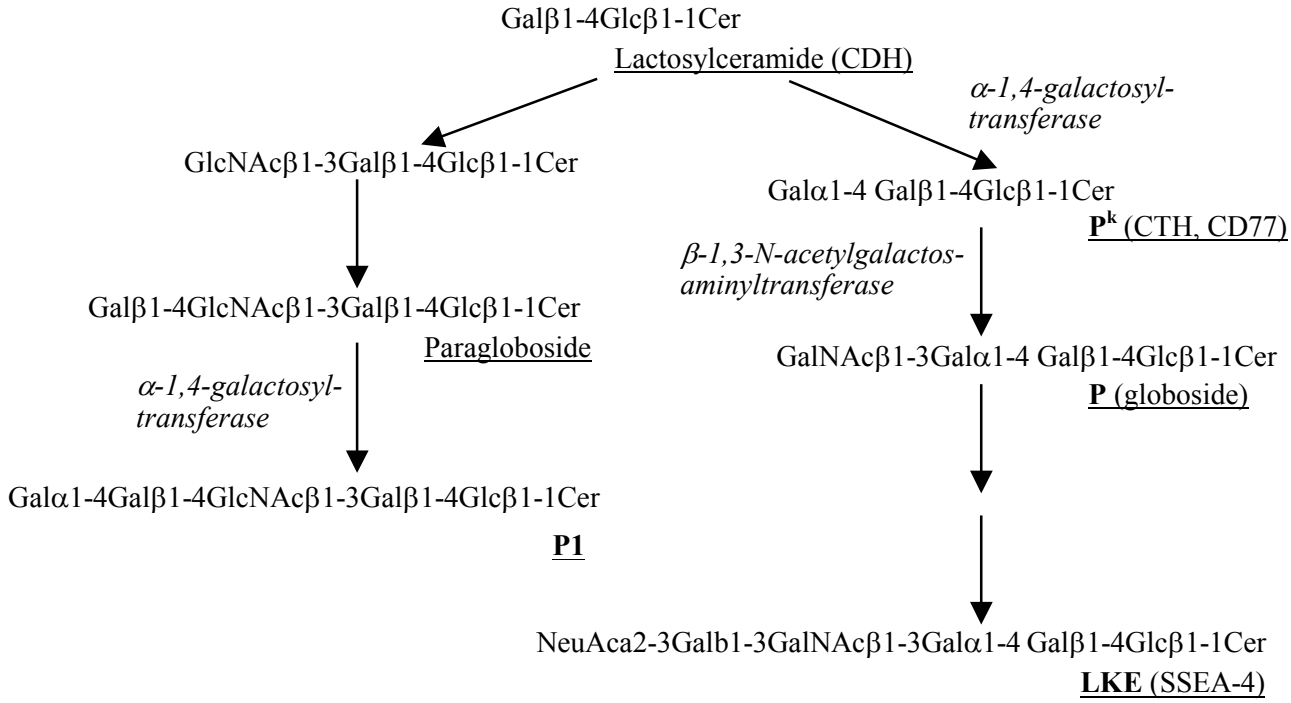

Fig. 1 Biosynthetic pathways involved in the formation of the P1, P, P^k, and LKE antigens

Phenotypes and Frequencies

Although only the P1 antigen is included in the P blood group system, the expression of the P and P^k antigen is usually discussed with the P1 antigen in the phenotypes of the P system because of the close relationship of the three antigens. According to the expression of these antigens, five phenotypes in the P blood group system are classified: P_1 (P1+P+P^k+), P_2 (P1–P+P^k+), P_1^k (P1+P–P^k+), P_2^k (P1–P–P^k+), and p (P1–P–P^k–). These phenotypes and their frequencies in different populations (8,9) are summarized in Table 1 (in the next page). As shown in Table 1, the frequency of the P1 antigen (the P_1 phenotype) in Taiwanese (9) is lower than those in Caucasians and in Blacks. From the P_1/P_2 phenotype frequency, the *P1+* gene shall have an approximate frequency of 18% and the *P1–* gene 82% in Taiwanese.

Table 1. Phenotypes and Frequencies of the P Blood Groups

Phenotype		Frequency		
		Caucasians	Blacks	Taiwanese
P_1	(P1+P+P^k+)	79%	94%	32%
P_2	(P1–P+P^k+)	21%	6%	68%
P_1^k	(P1+P–P^k+)	rare	rare	rare
P_2^k	(P1–P–P^k+)	rare	rare	rare
p	(P1–P–P^k–)	rare	rare	rare

Genetics of the P1, P, and P^k antigen Expression

Family study for the P1 antigen expression has located the *P1* locus on chromosome 22q11.2-ter. The *P1* gene probably encodes an α-1,4-galactosyltransferase, while the *P* gene, which has been shown to be independent of the *P1* locus, encodes a β-1,3-*N*-acetylgalactosaminyltransferase. Nevertheless, the relationship of *P1* and P^k genes, both producing α-1,4-galactosyltransferases, is undetermined, as two unusual phenomena in P1 and P^k antigen expression have been observed (3):

1. **Red cells of the individuals with the p phenotype lack P and P^k antigens and also lack P1 antigen.** Nevertheless, individuals with P_2 phenotype lack P1 antigen but express P^k and P antigens, and the P–P^k+ individuals may be P1+ (P_1^k phenotype) or P1– (P_2^k phenotype) (Table 1).
2. **No P_1 children were produced from the mating type P_2 X p** (none of the 37 children produced from 10 such matings had P_1 phenotype)**.**

Due to the observation of these phenomena, the relationship between the *P1* α-1,4-galactosyltransferase and the P^k α-1,4-galactosyltransferase has long been debated. Genetic Models for the P system have been proposed. Any genetic model must explain the two unusual phenomena mentioned above.

The Genetic Models Proposed

Three genetic models have been proposed to explain the expression of the P1 and P^k antigens.

1. **Two independent loci for P1 and P^k:** However, if two independent loci are involved, both of the *P1* and P^k loci must have mutational

change and thus leads to the p phenotype, or the *P1* and P^k loci are closely linked and the p phenotype results from deletion of the chromosomal region encompassing both loci.

2. **One *P1* locus with three alleles, *P1*, P^k, and *p*:** Naiki and Marcus (10) suggested that *P1* was a regulatory gene altering the acceptor specificity requirements of the P^k α-1,4-galactosyltransferase so that it could utilize paragloboside as well as lactosylcermide. *p* was an amorph.
3. **One *P1* locus with three alleles, P_1^k, P^k, and *p*:** Graham and Williams (11) proposed that P_1^k coding for an α-1,4-galactosyltransferase which can utilize both paragloboside and lactosylcermide as acceptor substrates, and P^k coding for an α-1,4-galactosyltransferase which can utilize only lactosylcermide, and *p* an amorph.

Recent Progress

In 2000, Steffensen *et al.* reported the cloning of the P^k α-1,4-galactosyltransferase gene (4). It was shown that the P^k α-1,4-galactosyltransferase could only utilize lactosylcermide as acceptor substrates to produce P^k structure but can not synthesize P1 antigen from paragloboside substrate. Furthermore, a single missense mutation in this P^k gene was identified in the individuals with p phenotype. Most interestingly, the P^k gene was found to locate on chromosome 22q13.2, which is within the region that has been predicted for the *P1* locus location. However, no polymorphism in the P^k gene was found to correlate with P_1/P_2 phenotype. This study reveals the P^k gene identity; however, still leaves the molecular genetic background for the correlation of the P1 and P^k antigen expression obscure.

研究目的、

Our goal in this research project is to establish the molecular genetics of the P blood group system. This includes **identification of the *P1* gene** and **elucidation of the molecular genetic basis explaining the correlation of the P1 and P^k antigen expression**. We will employ the positional cloning strategy and the SNP (single nucleotide polymorphism) information in NCBI (National Center for Biotechnology Information, U.S. National Library of Medicine) to localize the *P1* locus position.

文獻探討、

1. Landsteiner K, Levine P. Further observations on individual differences of human blood. Proc Soc Exp Biol NY 1927;24:941-942.
2. Sanger R. An association between the P and Jay systems of blood groups. Nature 1955;176:1163-1164.
3. Daniel G. Human Blood Group. Oxford, England: Blackwell Science; 1995.
4. Steffensen R, Carlier K, Wiels J, etc. Cloning and expression of the histo-blood group P^k UDP-galactose:Galβ1-4Glcβ1-Cer α1,4-galactosyltransferase. J Biol Chem 2000;275: 16723-16729.
5. Morgan WTJ, Watkins WM. Blood group P_1 substance. I. Chemical properties. Proc 9th Congr Int Soc Blood Transfus 1962:225-229.
6. Cory HT, Yates AD, Donald ASR, Watkins WM, Morgan WTJ. The nature of the human blood group P_1 determinant. Biochem Biophys Res Commun 1974;61:1289-1296.
7. Naiki M, Marcus DM. Human erythrocyte P and P^k blood group antigens: identification as glycosphingolipids. Biochem Biophys Res Commun 1974;60:1105-1111.
8. Reid ME, Lomas-Francis C. The blood group antigen factsbook. San Diego, CA: Academic Press; 1997.
9. Lin M, Broadberry RE. Immunohematology in Taiwan. Transfus Med Rev 1998;12:56-72.
10. Naiki M, Marcus DM. An immunochemical study of the human blood group P_1, P, and P^k glycosphingolipid antigens. Biochemistry 1975;14:4837-4841.

Graham HA, Williams AN. A genetic model for the inheritance of the P, P_1 and P^k antigens. Immunol Commun 1980;9:191-201.

研究方法、

As the obscure status between the *P1* and P^k genes, the positional cloning strategy is designed and will be used simultaneously to try to demonstrate the *P1* gene.

Five main steps are designed in our researches:

1. Assessing and collecting the typical pedigrees with the P_1/P_2 phenotypes
2. Pedigree analyses for localization of the position or the chromosome region of the *P1* gene candidate through the SNP information in human chromosome 22

3. Enzyme characterization of the *P1* gene product
4. Identification of the polymorphism in the *P1* gene correlating with the P_1/P_2 phenotypes and segregation analysis in the P_1/P_2 pedigrees
5. Further study required for elucidating the molecular genetics for the correlation of the *P1* and P^k genes

1. Assessing and collecting the typical pedigrees with the P_1/P_2 phenotypes

Assessing and collecting the pedigrees with the P_1/P_2 phenotypes is essential for further investigation. Through this procedure, research samples (genomic DNAs, B lymphocytes, *etc*.) from individuals with the P_1 and P_2 phenotypes can be gathered. Furthermore, the pedigrees are required for future segregation analysis for correlating any polymorphism identified in the cloned *P1* gene with the P_1/P_2 phenotype.

The P_1/P_2 phenotypes of the individuals assessed in this research will be determined by a standard serological procedure. As the phenotype distributions of P_1 and P_2 are 32% and 68%, respectively, in Taiwanese, it will not be difficult to obtain typical pedigree with P_1XP_2 parents and P_1 and P_2 siblings.

2. Pedigree analyses of SNP to localize the chromosome region of the *P1* gene candidate

The appropriate SNP positions have been selected through searching the SNP information of human chromosome 22 in NCBI, and PCR amplification and direct sequencing to analyze the SNP in the family members of the four P_1/P_2 pedigrees have been performed to test the feasibility of this strategy. Our preliminary results from segregation analysis agree with the previous data from family study for the P1 antigen expression, which shows the localization of the *P1* locus on chromosome 22q11.2-ter.

3. Enzyme characterization of the P1 α-1,4-galactosyltransferase

The protein product of the cloned *P1* gene candidate will be expressed through baculovirus expression in insect cells. The product will be purified and used in the *in vitro* glycosyltransferase assay. The enzyme character of the P1 α-1,4-galactosyltransferase will be analyzed to inspect its capability to synthesize P1 epitope from the paragloboside precursor. It is also important to inspect whether the enzyme expressed from the cloned *P1* gene has a broad specificity to synthesize P^k structure as well.

The reported P^k α-1,4-galactosyltransferase will also be expressed and prepared through baculovirus expression system in insect cells. Further enzyme characterization of the P1 α-1,4-galactosyltransferase and comparison with the P^k α-1,4-galactosyltransferase using different acceptor substrates will be proceeded. Establishment of this information is important in further realizing the relationship

between the P1 and P^k antigen expression.

However, if the cloned *P1* gene does not encode an α-1,4-galactosyltransferase, further study to demonstrate the role of the *P1* gene's product (a regulatory element for the P^k α-1,4-galactosyltransferase?) in producing P1 antigen will be designed and proceeded.

4. Identification of the polymorphism in the *P1* gene correlating with the P_1/P_2 phenotype and segregation analysis in the P_1/P_2 pedigrees

Demonstrating the correlation of the polymorphism in the cloned gene with the P_1/P_2 phenotype is essential to confirm that the gene identified from positional cloning or expression cloning is responsible for P1 antigen expression.

The *P1* gene of the P_2 individual will be analyzed and searched for any mutation (missense mutation, short nucleotide deletion or insertion, or gene deletion, *etc*.). Correlation of any identified mutation in the *P1* gene with the P_1/P_2 phenotype will be inspected, and segregation analysis for the mutant *P1* gene in the P_1/P_2 pedigrees will be performed.

Enzyme function analysis will be performed to demonstrate the activity of the enzyme expressed from the mutant *P1* gene through *in vitro* expression in the appropriate mammalian cell line if the correlated polymorphism identified in the *P1* gene is a missense mutation.

5. Further study required for elucidating the molecular genetics for the correlation of the *P1* and P^k genes

Identification of the *P1* gene is the first goal of this research project, and we are faithful in achieving this goal. We expect to further elucidate the molecular genetic basis of the relationship between the P1 and P^k antigen expression.

The reveal of the *P1* gene and the enzyme characters of the product encoded from the *P1* gene might provide helpful clue to explain the P1 and P^k antigen correlation.

討論

Questions and clues should be noted:

1. **If the *P1* and P^k locus are different, are they closely linked? How close they are? Are both of the genes mutated in the individuals with p phenotype?**
2. **Does the *P1* gene encode a regulatory element altering the acceptor**

specificity requirements of the P^k α-1,4-galactosyltransferase so that it could utilize paragloboside as well as lactosylceramide (as Naiki and Marcus proposed)?

3. **Does the *P1* gene encode an α-1,4-galactosyltransferase which can utilize both paragloboside and lactosylceramide as acceptor substrates (as Graham and Williams proposed)?**
4. **If the *P1* and P^k genes are identical, what kind(s) of factors might determine the P_1/P_2 phenotype? If this situation is true, positional cloning might reveal this information; however, expression cloning might fail to give any positive results.**

計畫成果自評

In this research project, we have successfully gathered twelve pedigrees with different P_1/P_2 phenotypes, and their genomic DNA samples have been prepared. The SNPs localized in the 5' –50 kb and 3' +20 kb regions of the P^k α-1,4-galactosyltransferase gene have been selected, and PCR amplification and direct sequencing to analyze the SNP in the family members of the four P_1/P_2 pedigrees have been performed. However, none of the SNP point has been demonstrated to correlate with the P_1/P_2 phenotypes. This result hampered us to perform further investigation to reveal the molecular basis for the P_1/P_2 phenotypes. The molecular basis for the P_1/P_2 phenotypes still awaits further elucidation.