# The P1PK blood group system: revisited and resolved

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This update on the P1PK blood group system (Hellberg Å, Westman JS, Thuresson B, Olsson ML. P1PK: the blood group system that changed its name and expanded. Immunohematology 2013;29:25–33) provides recent findings concerning the P1PK blood group system that have both challenged and confirmed old theories. The glycosphingolipids can no longer be considered the sole carriers of the antigens in this system because the P1 antigen has been detected on human red blood cell glycoproteins. New indications suggest that $P1P^k$ synthase activity truly depends on the DXD motif, and the genetic background and molecular mechanism behind the common $P_1$ and $P_2$ phenotypes were found to depend on transcriptional regulation. Transcription factors bind the $P^1$ allele selectively to a motif around rs5751348 in a regulatory region of *A4GALT,* which enhances transcription of the gene. Nonetheless, unexplained differences in antigen expression between individuals remain. ***Immunohematology* 2020;36:99–103.**

**Key Words:** P1, $P^k$, *A4GALT,* erythrocyte, blood group

After having expanded to include three antigens (P1, $P^k$, and NOR) and also having changed its name from the P blood group system to P1PK (since the P antigen resides in the GLOB system[1]), the status of this system has stabilized—although P1PK remained one of two systems for which a polymorphic blood group could not be genetically determined.[2] Thus, one of the major developments reported is the clarification of the genetic basis and underlying molecular mechanism explaining the presence ($P_1$ phenotype) or absence ($P_2$) of P1 antigen on red blood cells (RBCs).

## Antibodies

Although antibodies against the P1 antigen are usually not reactive at 37°C, exceptions occur. In 2016, anti-P1 alloimmunization in combination with an autoanti-I caused a delayed hyperhemolytic transfusion reaction in a pregnant patient with thalassemia intermedia.[3] When the implicated antibodies against P1 antigen are of immunoglobulin M (IgM) type, they risk being missed in antibody screening methods that primarily target IgG antibodies. This finding was well exemplified in a recent study involving a clinically mild acute hemolytic transfusion reaction caused by anti-P1.[4]

## Biochemistry

The enzyme specificity was further investigated, and it was shown that recombinant $P1P^k$ synthase uses both lactosylceramide and paragloboside as acceptor substrates to make $P^k$ and P1 antigens, respectively, and that the Gln211Glu switch indeed broadens its acceptor specificity to include Gb4 (Globoside, P) for formation of NOR antigen as well.[5] Furthermore, it had been debated for many years whether P1 occurs only as a glycolipid or also as part of glycoproteins. Studies supporting both ideas have been published.[6,7] In a recent study from our laboratory, the ability of $P1P^k$ synthase to use acceptors on both types of carriers was supported by the detection of P1 on human RBC glycoproteins. We also showed a dosage-dependent effect of *A4GALT* genotype on the level of P1 staining of RBC membrane proteins and, in addition, reported data supporting the idea that P1 appeared to be mainly carried on N-glycans in glycoproteins.[8] This notion was confirmed by the study of a recombinantly expressed catalytic fragment of $P1P^k$ synthase, which synthesized P1 structures on the N-glycans of saposin D.[9] It was also proposed that such P1-terminating N-glycans can serve as a decoy for Shiga toxins. Indeed, a method for in situ synthesis of the P1 pentasaccharide was developed by another group to be used as a ligand for neutralization of Shiga toxins.[10]

The crystal structure of the $P1P^k$ synthase, also known as 4-alpha-galactosyltransferase, has not yet been resolved. Jacob et al.,[11] however, demonstrated that disruption of the suggested DXD motif in this enzyme abolishes transferase activity. This finding indicates that the $P1P^k$ synthase constitutes a GT-A fold because the DXD motif is a common feature for these enzymes.[12]

Three different groups have performed genome-wide screens of Shiga toxin binding to a library of cells with each of the human protein coding genes knocked out by CRISPR/Cas9 (clustered regularly interspaced short palindromic

repeats with Cas9).[13–15] Using the toxin's specificity for the $P^k$ epitope, critical proteins for the antigen synthesis were identified and, in addition to known factors, such as those involved in the synthetic pathway of $P^k$, three genes stood out: *LAPTM4A, TM9SF2,* and *TMEM165*. The products of *TM9SF2* and *TMEM165* both seemed to affect the glycosphingolipid biosynthesis globally. Conversely, all three studies proposed the four-pass membrane protein, lysosomal-associated protein transmembrane 4 alpha, encoded by *LAPTM4A* as an activator of $P1P^k$ synthase. The product of a *LAPTM4A* mouse homologue has been suggested to be involved in intracellular transport of nucleosides,[16] which could indicate its role as a provider of activated sugars into the Golgi lumen.[13,14] Further work is needed to elucidate how *LAPTM4A* induces $P^k$ synthesis.

## Genetics and Molecular Mechanisms

### Null Alleles Resulting in p Phenotype

Currently, a total of 40 different alleles at the *A4GALT* locus have been reported to abolish the enzyme activity and cause the p phenotype; 37 are described on the homepage of the International Society of Blood Transfusion (ISBT; www.isbtweb.org) or at www.erythrogene.com.[17] Additionally, three null alleles with deletions in coding exon 3 were recently described.[18,19]

### Alleles Underlying the $P_1$ vs. $P_2$ Phenotypes

In the original review published in this journal,[1] theories on the molecular mechanism behind the two common phenotypes $P_1$ ($P^k$+, P1+) and $P_2$ ($P^k$+, P1–) were described, but no conclusive answers could be presented. Today, new insights have been made. In 2014, three novel *p* alleles were identified, all with large deletions in the 5´-end of the gene, spanning over exons 1 and 2a, and parts of intron 1, including the suggested promoter region and transcription binding sites. *A4GALT* transcripts were not detectable in these p samples, which supports the idea that the region is crucial for transcription of the gene.[20] The $P_1/P_2$-discriminating single nucleotide polymorphism (SNP) nucleotide 42 (C/T) located in exon 2a, also called rs8138197,[21] was further evaluated in 2014 by Lai et al.[22] In their study, two new SNPs, rs2143918 (G/T) and rs5751348 (T/G), were identified with a 100 percent correlation ($n$ = 338) to the $P_1/P_2$ phenotypes. Of the two SNPs, rs5751348G ($P^1$ allele) was crucial for increased *A4GALT* transcript levels, which is consistent with what had been found earlier for the $P_1$ phenotype.[21,23] The link between *A4GALT* and the P1 antigen has been further supported, since knockdown of the gene in primary erythroblasts abolishes the antigen.[24]

In addition to low levels of Lutheran blood group antigens, the In(Lu) phenotype shows lower than expected P1 expression. This finding was originally reported by Singleton et al.[25] to be due to heterozygosity for mutations in the *KLF1* gene, inactivating expression of the transcription factor Krüppel-like factor 1 (KLF1). Since our original review, this finding has subsequently been confirmed by several investigators, for instance, Kawai et al.[26] Nevertheless, the reason for weak P1 expression on In(Lu) RBCs has not been explained. Low levels or lack of P1 has been observed in individuals with $P^1$ genotypes and the In(Lu) phenotype in a study presented as a Congress abstract, in which *A4GALT* was proposed to be a target of KLF1.[24] Despite this tempting suggestion, knockdown of *KLF1* in cell lines did not alter the levels of *A4GALT* transcript in another study. However, the Runt-related transcription factor 1 (RUNX1) bound allele-specifically to $P^1$ alleles, and knockdown of its expression indeed lowered *A4GALT* expression based on transcription factor binding to a motif surrounding rs5751348G (Fig. 1).[27] Additionally, the transcription factor early growth response (EGR) family was proven to generate increased transcription of the $P^1$ allele compared with the $P^2$ allele and, more specifically, EGR1, active in erythroid cells, bound to $P^1$ alleles specifically and induced $P1P^k$ synthase expression (Fig. 1).[28] Although correlated to the $P^1/P^2$ genotype, the exposed P1 and $P^k$ antigen levels on the cell surface do vary within the genotype groups. The antigen quantity variations were discussed and evaluated both as outcome of molecular alterations as well as the cell membrane component (cholesterol) composition, but no further conclusions regarding additional genetic determinants to refine genotyping efforts could be made.[2]

## Disease Associations

P1 and $P^k$ antigens have previously been shown to have implications for disease susceptibility. In addition to the previously mentioned Shiga toxins, several pathogens can bind to the epitopes.[1] Furthermore, altered expression in cancerous tissue was already noted in the first patient identified to have the p phenotype.[30] New studies on both these subjects have been performed and some are summarized briefly here. In a series of papers, Jacob et al.[31] investigated possible roles for P1 and anti-P1 in patients with ovarian cancer. P1, $P^k$, and P antigens were all detected in ovarian cancer tissue, and

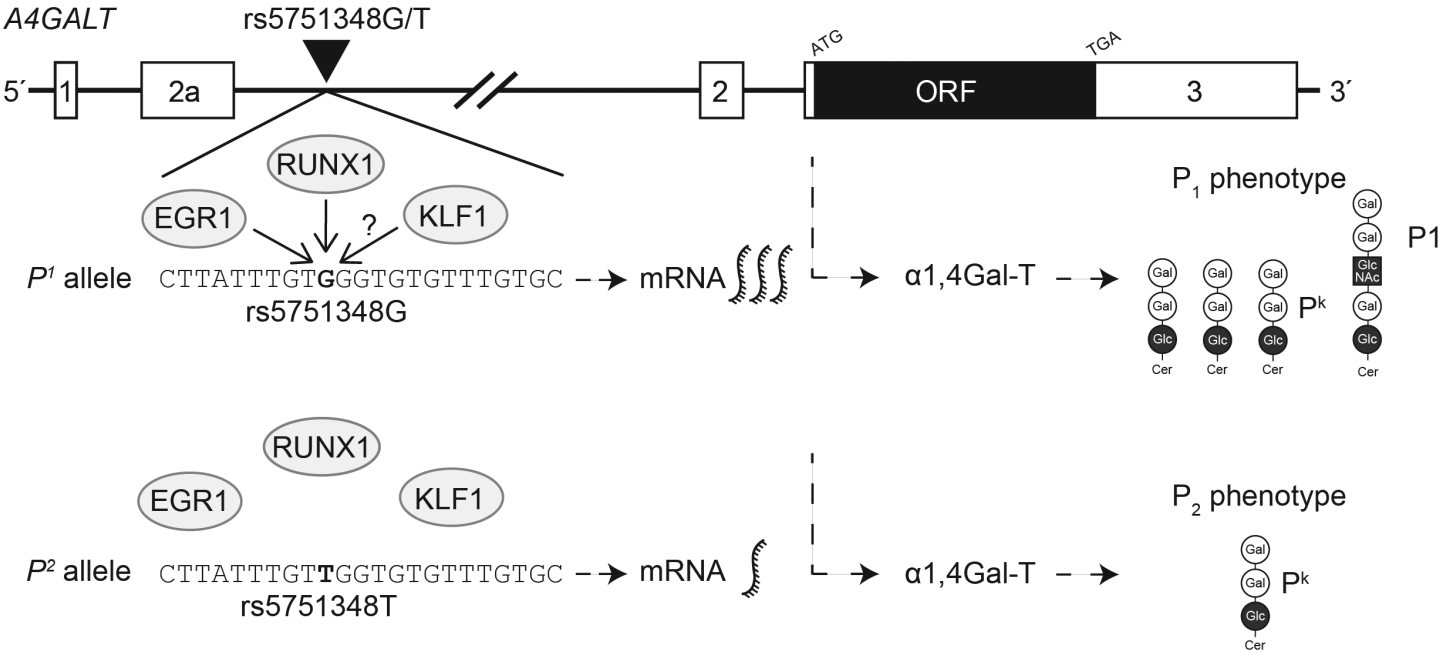


**Fig. 1** Transcriptional regulation determines the two major phenotypes in the P1PK blood group system, $P_1$ and $P_2$. A schematic illustration (not to scale) of the *A4GALT* gene coding for the $P1P^k$ synthase is shown. The single nucleotide variant rs5751348:G>T disrupts a transcription factor binding motif, since EGR1 and RUNX1 (and possibly KLF1) bind to the $P^1$ allele over the rs5751348:G and surrounding site, but have lowered affinity to the $P^2$ allele carrying the variant. The transcription factors binding to the $P^1$ allele enhance gene expression, resulting in increased levels of the $P1P^k$ synthase. The more abundant enzymes in $P_1$ individuals will then synthesize both the $P^k$ and P1 antigens, whereas the $P^2$ allele will only express enough $P1P^k$ synthase to make the $P^k$ antigen, and that to a lesser extent. EGR1 = early growth response 1; RUNX1 = Runt-related transcription factor; 1 KLF1 = Krüppel-like factor 1; Gal = galactose; Glc = glucose; GlcNAc = *N*-acetylglucosamine.

anti-P1 was found in ascites in comparable levels to those found in plasma. Among other findings, the group associated high P1 expression in IGROV1 cells, an ovarian cancer cell line, with elevated cell migration, but in further studies, deletion of *A4GALT* resulted in increased cell motility and invasiveness. Furthermore, E-cadherin–mediated cell–cell adhesion requires a functional $P1P^k$ synthase, and a lack thereof triggers epithelial-to-mesenchymal transition in cancer cells.[11,31,32]

$P^k$ was previously implicated to play a role in human immunodeficiency virus (HIV) infection. Initially, the antigen was recognized to facilitate HIV-host fusion through gp120 and CD4 interaction.[33,34] However, in T lymphocytes, $P^k$ is a minor component of the glycosphingolipid content, and in studies on lymphocytes from donors of p phenotype (lacking $P^k$), increased fusion susceptibility was detected compared with common cells.[33,35] Furthermore, high amounts of the $P^k$ antigen (as in the $P_1^k$ phenotype and Fabry disease) protect against HIV-1 (both R5 and X4 strains) infection *in vitro*.[35] In a clinical study by Motswaledi et al.,[36] individuals with the $P_1$ phenotype were associated with a higher rate of HIV infections when different blood group antigens were evaluated. Because it has also been shown that individuals of $P_1$ phenotype have higher $P^k$ expression on RBCs than $P_2$ individuals, these findings appear potentially contradictory, and additional work is required to understand how these blood groups affect pathobiology.

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