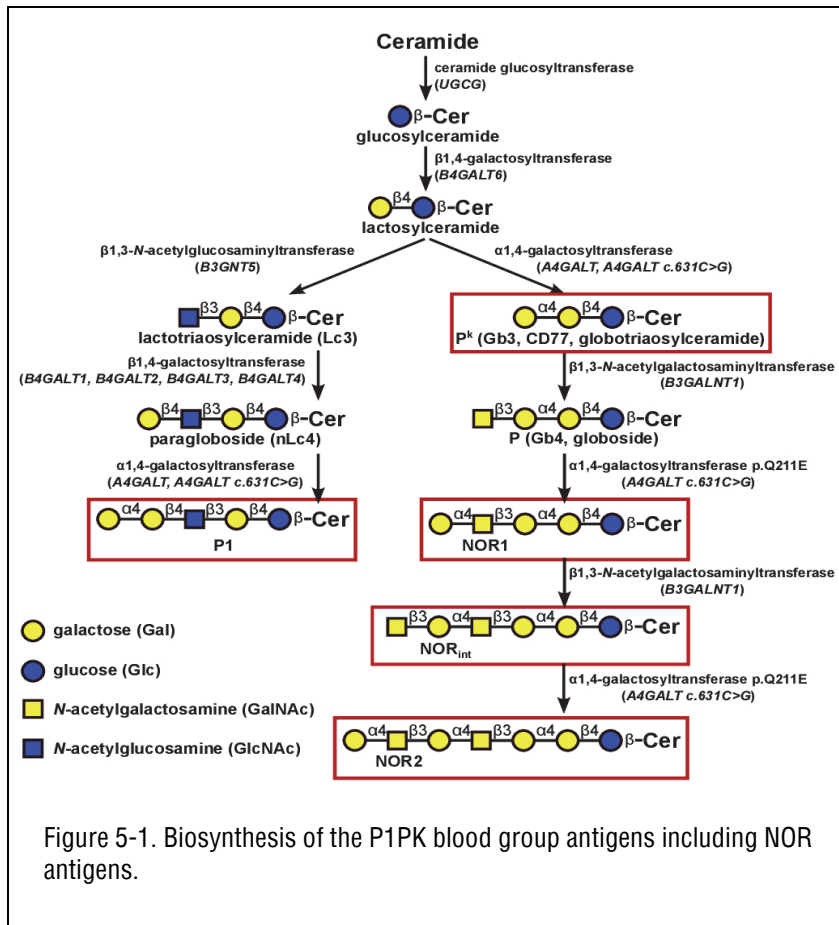


NOR Blood Group Antigen



NOR Antibodies

NOR antibodies occur in approximately 70% of human sera, and their titers vary significantly between individuals.⁵ The frequency of NOR antibodies is likely underestimated because they also occur in sera that do not agglutinate NOR-positive erythrocytes, probably due to low titers.⁸ Despite some similarity between NOR and the P₁ blood group antigen, anti-NOR titers were similar between P₁ and P₂ individuals.⁸ NOR antibodies do not cross-react with the Galα(1→3)Gal antigen.^{8,9} NOR antibodies react most strongly with Galα(1→4)Gal-

NAc β (1 \rightarrow 3)Gal, and over 100 times weaker with Gal α (1 \rightarrow 4)GalNAc. The antibodies also react with Gal α (1 \rightarrow 4)Gal and Gal α (1 \rightarrow 4)Gal β (1 \rightarrow 4)GlcNAc, which is why the NOR polyagglutination is inhibited by the P1 antigen.⁵ In contrast, anti-Gal α (1 \rightarrow 3)Gal antibodies react most strongly with Gal α (1 \rightarrow 3)Gal, and are very weakly inhibited by the NOR-related oligosaccharides.⁸ Similar antibodies have been found in horse, pig, and rabbit sera.¹⁰

The agent that elicits anti-NOR remains unknown. The Gal α (1 \rightarrow 4)GalNAc β 1 sequence capping the NOR glycosphingolipids was never before found in human or animal glycoconjugates, except in egg jelly coat mucin in *Rana ridibunda*.¹¹ The Gal α (1 \rightarrow 4)GalNAc β 1 units comprise internal portions of lipopolysaccharide O-specific chains of *Proteus mirabilis* and *Proteus vulgaris*,^{12,13} but NOR antibodies do not react with such lipopolysaccharides.⁵ However, similar structures may be present in intestinal microbiota as well as saprophytic and edible fungi (eg, lipids recognized by NOR antibodies were found in galactosaminogalactan from *Aspergillus fumigatus*¹⁴) and in some mushrooms (Lisowska E, personal communication).

A mouse monoclonal antibody nor1 18 was obtained by immunization of BALB/c mice with NOR-tri-HSA. This IgG1 antibody is highly specific for terminal Gal α (1 \rightarrow 4)GalNAc units, so it strongly reacts with NOR1 and NOR2 GSLs.¹⁵ The antibody agglutinates NOR-positive red cells, but it is a weak agglutinin, so detection requires performing the indirect antiglobulin test.

Molecular Background

The results of structural studies prompted the hypothesis that the NOR antigen must have emerged as a result of a mutation in one of the genes encoding glycosyltransferases. Analysis of multiple genes revealed that the NOR phenotype strictly correlated with a single nucleotide mutation (C>G) at position 631 in the *A4GALT* gene (NG_007495.2), which encodes the Gb3/CD77 synthase (α 1,4-galactosyltransferase, P1/P^k synthase, EC 2.4.1.228). The enzyme synthesizes the Gal α (1 \rightarrow 4)Gal moiety in the P^k and P1 blood group antigens.¹⁶ Further in-silico analysis revealed that the c.631C>G substitution (rs397514502) was a missense mutation leading to replacement of the glutamine at position 211 of the enzyme with glutamic acid (p.Q211E). Thirteen NOR-positive individuals from the TS family

NOR Blood Group Antigen

were heterozygous for the c.631C>G mutation.¹⁷ All 470 tested NOR-negative individuals were homozygous for C at this position. The same mutation (c.631C>G) was found in the American donor.¹⁷ When the cells of human teratocarcinoma cell line 2102Ep were transfected with vectors encoding the Gb3/CD77 synthase or its p.Q211E mutein, either transfectant expressed the P1 antigen, which is absent in nontransfected cells. In contrast, cells expressing the p.Q211E mutein produced both the NOR and P1 antigens.¹⁷ Thus, the c.631C>G mutation (rs397514502) in *A4GALT* alters the enzyme specificity, rendering it able to synthesize all three P1PK blood group system antigens: P^k, P1, and NOR. Using recombinant Gb3/CD77 synthase produced in insect cells, it was shown that the enzyme synthesizes both the P^k and P1 antigens, while its p.Q211E variant additionally synthesizes the NOR antigen. This was the first direct biochemical evidence that Gb3/CD77 synthase can synthesize two different glycosphingolipid antigens: P^k and P1, and, when the p.Q211E substitution is present, also produces the NOR antigen.¹⁸ Position 211 in the enzyme polypeptide plays a critical role in its specificity and activity, and substitution of Gln211 by other amino acids than Glu abrogates the enzyme activity.¹⁹ Presence of the c.631C>G mutation in *A4GALT* was associated with a slight decrease of the P1 antigen expression on human red cells.²⁰

The NOR Antigen and the P1PK Blood Group System

The human P blood group system was discovered by Landsteiner and Levine in 1927, when they showed that rabbits immunized with human red cells produced antibodies reacting with an antigen then named P and now called P1.²¹ The blood group system has since been renamed P1PK (International Society of Blood Transfusion system 003), and now consists of three glycosphingolipid antigens: P^k (Gb3, CD77), P1, and NOR.¹⁶ The molecular background of the P1PK blood groups system has been solved recently: it was found that RUNX1 and EGR1 transcription factors regulate expression of *A4GALT* gene by binding to a regulatory region in intron 1. The Presence of rs5751348[G] in the *P^l* allele enhances transcription, underlying the P₁/P₂ polymorphism.^{22,23} The P1PK blood system has been recently reviewed.²⁴