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DOCTORAL DISSERTATION

Changes in specificity of human α1,4-galactosyltransferase (Gb3/CD77 synthase): studies on the molecular background

ABSTRACT

Human α1,4-galactosyltransferase (Gb3/CD77 synthase, Pk synthase or P1/Pk synthase) is a glycosyltransferase that plays a central role in synthesis of glycosphingolipid antigens from three blood group systems: P1PK, GLOB and FORS. These blood group systems and the GLOB blood group collection represent a closely related set of thirteen glycosphingolipids. They are synthesized by the coordinated action of several glycosyltransferases, encoded by at least seven different loci. Three of these enzymes, including Gb3/CD77 synthase, show either different activity or different mRNA expression profile due to genetic polymorphisms, resulting in blood group diversity. P1PK has been the most elusive and is arguably the most complicated of 36 currently known blood group systems and its genetic background remains to be fully elucidated. Gb3/CD77 synthase transfers galactose residue to galactose of lactosylceramide, thus forming Pk (Gb3/CD77), a common antigen belonging to the P1PK system. It has long been thought that the same enzyme also synthesizes the P1 antigen, which contains the same terminal disaccharide (Galα1,4Gal), but all the available evidence, albeit strong, was inconclusive. In addition, a variant of this enzyme with p.Q211E substitution (caused by a single point mutation in A4GALT, c.631C>G, found in our laboratory) turned out to be responsible for the occurrence of the NOR antigen (the rarest of the three P1PK antigens, whose structure was also solved in our laboratory), terminating with an unusual Galα1,4GalNAc disaccharide. Results of this study pinpoint that c.631C>G mutation underlies the rare NOR phenotype and provide the first direct biochemical evidence that the consensus and the p.Q211E variant enzyme are able to synthesize Pk and P1or Pk, P1 and NOR antigens, respectively. Thus, Gb3/CD77 synthase is the first described glycosyltransferase in which a point mutation changes the acceptor specificity from galactose to galactose/N-acetylgalactosamine.