

MOLECULAR GENETICS OF HYPERBILIRUBINEMIA AND DEVELOPMENT OF MOLECULAR SCREENING TEST FOR SEVERE NEONATAL JAUNDICE

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INTRODUCTION

Neonatal jaundice is the most common clinical condition affecting newborns. Severe neonatal jaundice poses a direct threat to the brain and is associated with significant neonatal morbidity. In Malaysia, 75% of newborns are jaundiced in the first week of life and 25-30% of these patients develop severe jaundice. In 40% of these cases, the underlying causes have not been identified and genetic causes have been implicated.

OBJECTIVE

This study aimed to develop rapid molecular screening assays to identify genotypic variations in the red blood cell Glucose-6-phosphate dehydrogenase (G6PD) gene, Uridine 5' diphosphate glucuronosyl transferase 1A1 (UGT1A1) gene, a key enzyme in bilirubin metabolism and gene for the Organic Anion Transport Polypeptide 2 (OATP 2) a transport protein thought to be responsible for uptake of bilirubin from the blood into the liver and the study aimed to determine whether variants of these three genes are independent genetic risk factors associated with severe neonatal hyperbilirubinemia.

METHOD

We first developed various quick PCR-based assays to detect the range of known mutations in all the three genes. Taqman Minor Groove Binder (MGB) dual labeled probe assay was established to genotype G6PD mutations c.1376G>T, c.1388G>A, c.1024, c.95A>G, c.871G>A, c.563C>T, c.487G>A and c.392G>T. Two genotyping plates were developed in order to screen the eight most common genotypes in our Chinese and Malay populations. Taqman MGB dual labeled probe assay was also established to detect variant c.211G>A of the UGT1A1 gene and variant c.571T>C and c.597C>T of OATP1B1 gene. High Resolution Melting (HRM) analysis was developed for the detection of variants c.388A>G, c.521T>C and IVS5 – 107_112 6bp deletion of the OATP1B1 gene. This project involved two study populations. The first, a cross-sectional study involved 74 Chinese term infants with severe hyperbilirubinemia (SB \geq 300 μ mo/L) and 125 healthy term infants without severe hyperbilirubinemia in a referral medical centre. The second was a study on a cohort of 333 neonates (53 term

infants with hyperbilirubinemia; PTSB \geq 250 μ mol/L and 280 healthy term infants) in a state hospital. DNA was extracted from cord blood or venous blood and subjected to various Real-Time PCR-based assays that have been established. All samples were subjected to DNA sequence analysis for exons 2 – 13 of the G6PD gene, exon 1 of the UGT1A1 gene and exons 4 and 5 of the OATP2 gene.

RESULTS

We were able to isolate DNA from dried blood specimens stored at ambient temperature up to one year. All results from genotyping methods were in complete concordance with the DNA sequencing results. G6PD genotyping plates were able to detect more than 90% of the variants of G6PD mutations in local populations. Results on the Chinese neonates showed that G6PD mutations ($P < 0.0001$) and UGT1A1 c.211G>A variant ($P=0.04$) were significantly more common in the hyperbilirubinemic group. The incidence of homozygous c.211G>A UGT1A1 variant was significantly higher in hyperbilirubinemic newborns when compared to the normal neonates ($P < 0.001$). None of the five polymorphic variants of OATP2 gene were found to be significant risk factors to development of severe hyperbilirubinemia. The incidence of c.571T>C however was higher in the normal Chinese compared to the neonates with severe jaundice (50% vs 30.8%; $p=0.01$). Forward logistic regression analysis showed that the only significant genetic risk factor in Chinese infants was homozygous 211 variant of the UGT1A1 gene (adjusted OR = 37.7, 95% C.I.: 4.4, 324.1; $p=0.001$). The significance of findings from the second study were seen in the Malays neonates (40 infants with hyperbilirubinemia and 180 healthy term infants). G6PD mutations (14.6% vs 4.3%; $p=0.023$) and IVS5 – 107_112 6bp deletion of the OATP2 gene ($p=0.034$) was significantly higher in the hyperbilirubinemic Malays newborns. Homozygous variant c.571T>C was significantly higher in the normal Malay newborns compared to the neonates with PTSB>250 μ mol/L (10.7% vs 0%; $p= 0.045$).

CONCLUSION

This study showed that homozygous variant c.211G>A of UGT1A1 gene is an independent genetic risk factor for severe neonatal jaundice. G6PD mutations and OATP2 gene variants of IVS5 – 107_112 6bp deletion are shown to be strongly associated with neonatal hyperbilirubinemia. These findings and the development of useful diagnostic assays would contribute to improvement of strategy for prevention of severe neonatal jaundice and kernicterus. The new knowledge on the association of OATP2 gene variants with hyperbilirubinemia appear to support the notion of its role in bilirubin uptake and warrants further study to elucidate its precise role.