Advances in studies on the polymorphism of UGT1A1 gene in neonates with unexplained hyperbilirubinemia

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Abstract: Neonatal hyperbilirubinemia is a common clinical symptom in the neonatal period. Severe jaundice can cause neonatal bilirubin encephalopathy. Clinically because severe hyperbilirubinemia more causes, the cause of hemolysis and infection is more common, this also accounted for a certain proportion of unknown reasons. UGT1A1 is a key enzyme for bilirubin metabolism, and unexplained jaundice is associated with a decrease in UGT1A1 activity caused by mutations in UGT1A1 gene, leading to neonatal persistent jaundice. The relationship between neonatal unexplained hyperbilirubinemia and UGT1A1 gene polymorphism was reviewed.

Key words: UGT1A1 gene polymorphism; Crigler-Najjar syndrome; Gilbert syndrome; neonatal hyperbilirubinemia

Preface

Neonatal jaundice is the most common clinical neonatal disease, about 60% of newborns can occur in varying degrees of jaundice [1], neonatal jaundice incidence and severity in Asia and Indians in the more obvious, in the Caucasus The lower incidence of race [2]. Most of the clinical physiological jaundice can naturally subside, but there are still some part of the pathological jaundice delayed healing, severe cases can cause high bilirubin encephalopathy. Urinary uridine diphosphate glucuronosyltransferase (UGT1A1) is a key enzyme in bilirubin metabolism, UGT1A1 gene mutation can cause UGT1A1 enzyme activity decreased to varying degrees, unexplained jaundice and UGT1A1 gene polymorphism, in order to prevent neonatal hyperbolic Erythropoiesis caused by irreversible damage, clear neonatal hyperbilirubinemia etiology and pathogenesis and UGT1A1 gene polymorphism has an important clinical significance [3-5].

1 Etiology and pathogenesis of neonatal hyperbilirubinemia

Neonatal jaundice is divided into physiological and pathological jaundice, the current domestic standard, neonatal physiological jaundice, the total bilirubin level is less than 12mg ≈ DL-1, pathological jaundice are mostly 12 ~ 15mg ≈ DL- 1, or even higher level [6]. Neonatal pathology hyperbilirubinemia etiology of complex and diverse, the main causes include; ABO blood group incompatibility, Rh maternal and child blood type hemolytic disease, G6PD enzyme deficiency and other red blood cell diseases caused by hemolytic jaundice; respiratory infections, urinary tract infection As well as sepsis and other red blood cell damage caused by excessive infection jaundice; intracranial hemorrhage, skull hematoma, visceral bleeding and other bleeding caused by extravascular hemolysis; erythrocytosis, hypothyroidism or abnormalities, breast milk jaundice, meconium discharge delay, lack of feeding or abnormal decline in body mass or dehydration, and other unexplained several categories; of which the highest proportion of infectious and hemolytic, respectively, about 39.9% and 19.9%, unexplained hyperbilirubinemia also accounted for a certain proportion, about 26% [6]. In neonatal hyperbilirubinemia, infectious, hemolytic and unexplained jaundice is the three major causes of hyperbilirubinemia, and clear etiology can effectively control neonatal hyperbilirubinemia, so as to avoid high Bilirubinemia caused by bilirubin encephalopathy, and unexplained jaundice, because of its reasons are not clear, the clinical diagnosis and treatment of neonatal unexplained hyperbilirubinemia has brought some difficulties.

Unexplained jaundice is associated with UGT1A1 gene polymorphism. Normal human blood non-binding bilirubin and albumin binding, transported to the surface of liver cells, liver cells uptake, and transported to the liver microsomes, UGT1A1 enzyme in the role of glucuronic acid and water-soluble bilirubin, From the liver cells to the capillary bile duct, and excreted through the digestive tract to the body, but UGT1A1 gene mutation can cause UGT1A1 expression decreased UGT1A1 enzyme activity decreased to varying degrees, resulting in non-binding bilirubin in the blood cannot be combined with normal bilirubin exclusion in vitro, leading to varying degrees of hyperbilirubinemia occur [7].

2 Neonatal unexplained hyperbilirubinemia and UGT1A1 gene mutation related diseases

UGT1A1 gene polymorphism can cause UGT1A1 gene expression decreased, resulting in UGT1A1 enzyme activity decreased to varying degrees, causing non-binding bilirubin levels increased in varying degrees, such as related gene disease Gilbert syndrome and Crigler-Najjar syndrome. Gilbert syndrome is a kind of liver without organic disease and non-binding bilirubin slightly elevated as the main performance of autosomal genetic disease, in the past that the disease is very rare, but in recent years, with the continuous development of medical technology , The incidence of the population of 3% to 12%, especially in adults more common (18 to 30 years old), male to female ratio of 10 ~ 1, the UGT1A1 activity of normal 30%, non-binding bilirubin level often In the 17 ~ 50μmol·L-1 (60μmol·L-1) the following [8], children's growth and development and physiological function had no significant effect, the clinical temporary treatment.

Crigler-Najjar syndrome is due to the genetic defect of UGT1A1 leading to partial or complete disorder of unbound bilirubin glucuronidation process, including Crigler-Najjar syndrome type I and type II [9]. Crigler-Najjar syndrome type I is a serious reduction or even deficiency of UGT1A1 enzyme, manifested as severe hyperbilirubinemia, serum non-binding bilirubin levels in 342 ~ 684μmol·L-1, p-phenobarbital and other drugs And blue light treatment is invalid, easy to lead to bilirubin encephalopathy, poor clinical prognosis, the best treatment for liver transplantation; Crigler-Najjar syndrome type II is a significant reduction of glucuronuclease but not disappear, the UGT1A1 activity is normal 10% [10], the degree of jaundice than the former light, serum non-binding bilirubin levels in the 103 ~ 342μmol·L-1, drug treatment of phenobarbital induced a certain effect, the prognosis is relatively good [11-12].
3 UGT1A1 gene structure and its polymorphism

UGT1A1 gene polymorphism can lead to UGT1A1 gene expression decreased, and cause UGT1A1 enzyme activity decreased to varying degrees. The UGT1A1 gene is located on the human chromosome 21 long arm 37 region 8 band (2q37 8), which is composed of five exons, including the first exon A1 and four common exons (2-5). UGT1A1 gene is composed of promoter and coding region, the promoter is a non-coding region, located in the upstream regulatory region, but regulatory gene expression, UGT1A1 gene polymorphism mainly in the coding region, including missense mutation, insert mutation and nonsense mutation. In addition to mutations can occur in the promoter region, exon and intron splice sites. Canu et al [13] reported that there are currently found more than 130 kinds of UGT1A1 gene mutation, single nucleotide substitution mutation of 91 species (14 kinds of nonsense mutation and 77 missense mutation), single nucleotide deletion of 21 species. There were 10 single nucleotide insertions and 8 mutations in the promoter and intron.

TATA box promoter is the transcription initiation point of UGT1A1 gene, located at 23 ~ 38bp, which is the binding site of transcription factor IID (TFIID), which regulates the transcription initiation of DNA, and the increase of TA sequence can make TATA binding protein The affinity of the TATA box was reduced, resulting in a decrease in UGT1A1 gene expression. Normal UGT1A1TATA genotype was homozygous for 6 repeats of TA base A (TA) 6TAA (6/6). The two genotypes of the mutant were 7 repeats of TA base A (TA) 7TAA (7/7) (6/7) mutant heterozygote, compared with the wild type, TATA mutant homozygous, TATA mutant heterozygote to the liver tissue UGT1A1 enzyme activity Down 52% and 37% [14]. The incidence of synovial bilirubin in synovial homozygous TA7 was higher than that in TA6 / 7 and TA6 / 6, and the incidence of TATA box polymorphism in different regions and populations was different, the highest frequency in the African region was 49.5% While Europe followed by 38.7%, 16.0% in Asia [15-16]. However, mutations in the TATA box in the African region can reduce the risk of neonatal hyperbilirubinemia, suggesting that there are other factors that reduce the risk of hyperbilirubinemia in the African region, such as long exposure time [17]; In the Asian region, the frequency of the first exon G71R mutation, the 211 gene from G mutation to A, 71 amino acids from glycine into arginine, making UGT1A1 gene expression decreased, UGT1A1 enzyme activity decreased, In the Caucasian and African races, this mutation has not been found, and the frequency of mutation in the East Asian population (Korea, Japan, Taiwan, Malaysia) is 16% to 26% [18]. Akaba et al [19] study showed that UGT1A1 gene exon G211A missense mutation in Japanese neonatal hyperbilirubinemia high incidence of the need for phototherapy in children with a frequency of 0.47, significantly higher than (0.16); Prachukthum et al [20] found that, G71R mutation in the high bilirubin group and the control group were 0.15 and 0.04, respectively, indicating that G71R is a high risk factor for hyperbilirubinemia in Thailand; Muslu et al [21], Huang et al [22] Malaysia and China Taiwan population study found that G71R is a Taiwanese population of hyperbilirubinemia risk factors; Zhong Dani et al [23] found that China's Guangxi hyperbilirubinemia children with G211A allele frequency of 0.21, Normal healthy children was 0.083, the difference was statistically significant. G71R homozygous mutations increase the incidence of hyperbilirubinemia and increase the level of total bilirubin, leading to severe hyperbilirubinemia. Long et al [24] Meta-analysis concluded that mutation in the G71R locus in Asia is a high risk factor for neonatal hyperbilirubinemia, but there is no conclusion in the Caucasian race, whereas in the Asian region, the promoter TATA box mutation has not yet been That is the neonatal hyperbilirubinemia factors in the Caucasian population is a controversial factor, pending further study.

There are more mutations in the UGT1A1 gene in the Asian region. G493R, P364L, g. (3) reported that the mutation point of UGT1A1 gene in 211 cases of Crigler-NajjarII syndrome in China was 211G> A (G71R), c. The mutation of UGT1A1 gene was found in 1 case of Crigler-NajjarII syndrome in China. The mutation of UGT1A1 gene was 211G> A, G71R, 508_510delTTC (p.F170-) and c. 1456T> G (Y486D); Tiwari et al. [25] reported that 211G> A (G71R), g. -327T> G, TATA box is a risk factor for neonatal hyperbilirubinemia; Ko et al [26] reported that 5 cases of Crigler-Najjar syndrome detected G71R mutation and Y486D complex mutation; Wanlapakorn et al [27] Reported that one case of the diagnosis of Crigler-Najjar syndrome in Thailand, the genetic mutation point for the first exon 558C> A homozygous mutation, the parents of this site heterozygous mutant carriers. In China and other Asian regions, the mutations associated with the Gilbert syndrome are mainly G71R, P229 and Y486D mutations located in the exon, whereas in the Caucasian and African races, GS-related mutations are mainly TATA. Yamagoto et al. [28] showed that the G71R homozygous model UGT1A1 activity was normal (32.2 ± 1.6%), G71R heterozygous model activity was normal (60.2 ± 3.5%), Y486D The activity of homozygous model was normal (7.6 ± 0.5%), and the activity of G71R and Y486D model was normal (6.2 ± 1.6%).

4 G6PD enzyme deficiency, breast milk jaundice and UGT1A1 gene polymorphism

Breast milk jaundice is one of the causes of neonatal jaundice, Shibuya et al [29] studies have shown that unsaturated fatty acids in breast milk, especially oleic acid, linoleic acid and docosahexaenoic acid (DHA), inhibit human UGT1A1 In vivo expression of activity, and Aoshima et al [30] that supplements glucose can become a neonatal breast milk jaundice treatment, adequate calorie intake can induce UGT1A1 gene in the small intestine full expression, thereby reducing serum bilirubin levels, In the case of glucose supplementation, continuous breastfeeding may be allowed. Yang [31] and other reports, in breast milk neonatal jaundice patients, G71R point mutation is a high risk factor for hyperbilirubinemia; Maruo et al [32] studies have shown that in East Asia, delayed breast milk jaundice, Half of the breast milk jaundice for homozygous G71R mutation, and its serum bilirubin levels higher than other types of mutations, thus confirming G71R mutation is a persistent risk of breast milk jaundice. However, recent studies [33] found that the mutation in the TATA promoter box was a protective factor for breast milk jaundice, and that of the TATA mutation was lower.

In China's Guangdong, Guangxi, Jiangxi, Hunan, G6PD deficiency caused by the lack of broad bean disease is more common, and neonatal jaundice, G6PD deficiency caused by hemolytic jaundice is one of the main reasons, G6PD deficiency is one of the most common X chain Which is the most common hereditary enzyme disease, but also G6PD gene mutation, the main mutation characteristics of point mutations in our population to G1388A, G1376T, A95G the three most common mutation, while the lack of G6PD neonatal hyper bilirubin The probability of hemolytic disease is much higher than G6PD normal [34]. In the absence of G6PD neonatal severe hyperbilirubinemia, usually combined with UGT1A1 gene in the promoter or coding region of the mutation, G6PD

Gene mutation and UGT1A1 gene mutation major caused by the degree of neonatal jaundice severely. In the study of G6PD-deficient severe hyperbilirubinemia [35], G71R mutation in the experimental group was found to be higher than that in the control group, with a mutation rate of 55.1% and 25.0%, respectively,
indicating that G71R Site mutation is a risk factor for severe hyperbilirubinemia in patients with G6PD deficiency. Fu Wenping et al [36] found that TATA box mutation was not associated with neonatal jaundice and G6PD deficiency in Guangxi; Zahedpasha et al. [37] also found that promoter polymorphism was not associated with G6PD deficiency.

5 Detection of UGT1A1 gene polymorphism in neonatal unexplained hyperbilirubinemia

Neonatal unexplained hyperbilirubinemia commonly used clinical test methods, including liver function, low calorie test, phenobarbital experiments, and its closely related to the UGT1A1 gene polymorphism of more than 130 kinds of mutations, related to the Congenital non-hemolytic disease with Gilbert syndrome and Crigler-Najjar syndrome, how to detect these mutations is the scientific research and clinical urgent need to solve the problem. Gene sequencing is the gold standard for the diagnosis of genetic diseases. At present, there are gene chip technology, real-time fluorescence quantitative PCR technology and high-resolution melting curve technique. Gene sequencing is the most commonly used method to extract the whole blood of patients, according to the need to detect the UGT1A1 gene mutation point design primers, DNA as a template for PCR direct sequencing [38]. Gene chip technology based on the principle of base pairing principle, according to the need to detect the mutation point of the design of primers and probes, the probe fixed in a specific area of ??the chip to form a DNA micro-queue, the patient's whole blood extraction DNA amplified PCR The product was matched with the probe of the chip by base pairing. The signal was captured by chemical luminescence, chemical fluorescence or isotope labeling, and the signal strength was analyzed by computer to find the site of mutation [39]. Real-time fluorescence quantitative PCR technology principle is to add in the common PCR system with a fluorescence group TaqMan probe, the use of fluorescence signal accumulation detection of the entire PCR reaction process, the probe is an oligonucleotide, both ends of a report And the quenching of the fluorescent group, the probe is bound to any single strand of DNA, and the 5 'end -3' end exonuclease activity of the Taq enzyme is enzymatically digested with the PCR amplification, So that the fluorescent group and the quenching of the fluorescent group separation, so that the fluorescence monitoring system can receive a fluorescent signal, that is, each amplification of a DNA chain, there is a fluorescent molecule formation, to achieve the accumulation of fluorescence signals and PCR products formed completely And the fluorescence signal was analyzed by ABI system, and the gene mutation point was obtained. DNA melting curve technique is to insert DNA with double-stranded DNA, and according to the difference between the DNA content of the DNA sequence and the base, the double-stranded DNA cannot be complementary to each other during the PCR amplification, so that the fluorescent dye Released from the DNA of the chain, and the curve obtained by collecting the intensity of the fluorescence signal was compared with the standard curve to determine whether there was a mutation [40]. It is not yet widely used in clinical practice, but the current real-time fluorescence quantitative PCR technology due to high sensitivity, high specificity, high throughput, etc., because of the advantages and disadvantages of high efficiency, high specificity, and high throughput and so on. In the clinical laboratory gradually applied, is expected to become widely used in clinical detection of gene mutation in a new method [41].

Neonatal hyperbilirubinemia is the most common neonatal physiological and pathological phenomena, the general pathological jaundice if you can remove the cause in time to give Blu-ray and other related treatment, the effect is better, jaundice can be reduced to normal; but unexplained Jaundice, especially persistent jaundice and severe hyperbilirubinemia, is the cause of neonatal morbidity and mortality increased a large 'silence' factors in the clinical diagnosis and treatment is still some difficulties. At present, domestic and foreign research mainly through the general population to understand UGT1A1 gene polymorphism, neonatal unexplained hyperbilirubinemia, UGT1A1 gene polymorphism less, so the neonatal unexplained hyperbilirubinemia Patients, study UGT1A1 gene polymorphism and genetic analysis, to clear neonatal unexplained hyperbilirubinemia is important. Therefore, the relationship between UGT1A1 gene polymorphism and neonatal unexplained, it need to further studies.

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